

From social experience to social behaviour: hormonal and behavioural phenotypes during adolescence in male guinea pigs

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CRedit authorship contribution statement

Melanie Gleske: Methodology; writing – original draft; investigation; formal analysis; visualization; data curation. **Carolin Munding:** Formal analysis. **S. Helene Richter:** Conceptualization; writing – review and editing. **Sylvia Kaiser:** Conceptualization; methodology; supervision, writing – review and editing; funding acquisition. **All authors critically revised the manuscript and gave final approval for publication.**

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Ethics Statement

All procedures complied with the regulations covering animal experimentation within Germany (Animal Welfare Act) and the EU (European Communities Council Directive 2010/ 63/ EU), and were approved by the local and federal authorities (Landesamt für Verbraucherschutz und Ernährung Nordrhein-Westfalen “LAVE (ehemals LANUV) NRW”, reference number: 81-02.04.2022.A080).

Conflicts of Interest

The authors declare no conflict of interests.

Data availability

Open data and code are available via the Open Science Framework (OSF): <https://osf.io/k9s5r/overview>.

Abstract

Adolescence is the transition from juvenility to adulthood and is characterised by prominent endocrine, neural and behavioural alterations. Thus, adolescence represents a sensitive phase during which social experiences can shape endocrine and behavioural phenotypes. Although the influence of the social environment during adolescence has been widely investigated, most studies assessed such effects only before and after a given social experience, providing limited insight into how these phenotypes change within this ontogenetic phase. Our goal was therefore to examine potential effects of different social environments on the endocrine and behavioural phenotype in male guinea pigs by repeatedly assessing them during adolescence. For this approach, twenty domestic adolescent male guinea pigs (*Cavia aperea* f. *porcellus*) were housed in two distinct social environments: while males of both groups lived in heterosexual pairs, males of one group additionally received regular social stimulation through repeated short encounters with unfamiliar conspecifics, whereas males of the other group did not. This procedure increased the frequency and diversity of social interactions. Hormone concentrations and behavioural parameters were assessed repeatedly throughout the experiment. We hypothesised males from the two social conditions to differ in their hormonal and behavioural phenotype across adolescence. The difference in baseline cortisol concentrations between the social conditions changed over time. In addition, repeatability analyses revealed social condition-specific patterns in different components of cortisol responsiveness, suggesting that adolescent social environments may not only influence endocrine phenotypes themselves but also the stability of inter-individual differences in these traits. Males with additional social stimulation showed increased sociopositive and courtship behaviour across social contexts, which may reflect social niche conformance to a socially more variable and less predictable environment. Taken together, these findings show that social experiences during adolescence can shape endocrine and behavioural phenotypes in male guinea pigs, but that these adjustments may differ in their temporal dynamics.

Keywords

Basal cortisol, basal testosterone, cortisol responsiveness, social environment, social niche conformance, sociopositive behaviour

1. Introduction

The social environment of an individual is defined by its conspecifics and their interactions with the focal individual (Formica & Tuttle, 2009; M. I. Kaiser et al., 2024; Saltz et al., 2016). These social interactions can shape the individual's behavioural development profoundly (Prounis et al., 2015; Zimmermann, Kaiser, & Sachser, 2017). Most research on the development of behavioural phenotypes has focused on early life phases, particularly the time from birth until weaning (Champagne, 2013; Champagne & Curley, 2005; Lukas et al., 2011; Prounis et al., 2015; Sachser et al., 2013). During this time, neural circuits are highly plastic and the organism is peculiarly susceptible to external influences from the (social) environment (Champagne & Curley, 2005; Sachser et al., 2013), which are characteristics defining early life as a sensitive developmental phase (Fawcett & Frankenhuis, 2015). As shown in different rodent species, social environmental cues experienced by the mother can be transmitted to the offspring from conception to weaning, thereby shaping the offspring's phenotype (Brunton & Russell, 2010; Brust et al., 2015; Coutellier et al., 2008; S. Kaiser & Sachser, 2005; Macri & Würbel, 2006). However, in recent years it has become increasingly clear that adolescence is another sensitive phase during which behavioural trajectories can be shaped or reshaped in response to the social environment (Burke et al., 2017; Fuhrmann et al., 2015; Sachser et al., 2018, 2020).

Adolescence is the ontogenetic phase encompassing the gradual transition from juvenility to adulthood (Spear, 2000). In this phase, prominent alterations in the endocrine system, neural circuitry and behaviour occur (Romeo et al., 2002; Yurgelun-Todd, 2007). Even though juveniles are nutritionally independent of their parents after weaning, offspring of several mammalian species continue to display filial attachment beyond weaning (Hennessy et al., 2003). During adolescence, individuals gradually become socially independent from their parents, often disperse from their natal social environment and encounter unfamiliar conspecifics (Cryns et al., 2022; Lin & Wilbrecht, 2022). As sexual maturity emerges during adolescence, social interactions with conspecifics increasingly occur in a reproductive context (Zimmermann, Kaiser, Hennessy, et al., 2017; Zimmermann, Kaiser, & Sachser, 2017). Consequently, adolescence is often characterised by major changes in the social environment, requiring individuals to adjust their behavioural phenotype to the prevailing social conditions (Ruploh et al., 2014). This is, for example, well-illustrated in guinea pig males (*Cavia aperea* f. *porcellus*), which can develop distinct behavioural and reproductive strategies when living in differently sized groups during adolescence. Males reared in large mixed-sex groups, i.e., under high population density, usually display low-aggressive queuing strategy, whereas males reared in heterosexual pairs, i.e., under low population density, develop a high-aggressive resource defense strategy (Mutwill et al., 2020; Sachser et al., 2013, 2018; Zimmermann, Kaiser, & Sachser, 2017). The queuing strategy is thought to be adaptive under socially complex conditions, because it allows males to avoid reproductive competition until they are

large enough to effectively challenge dominant males for mating access (Sachser et al., 2018; Zimmermann, Kaiser, & Sachser, 2017). In contrast, the resource defence strategy may increase reproductive success under low-density conditions by allowing males to monopolize access to their female partner (Mutwill et al., 2020; Sachser et al., 2013, 2018). Similar patterns have also been reported in zebra finches (*Taeniopygia guttata*), where males reared in socially more complex environments during adolescence display lower aggressiveness and courtship behaviour than males reared with a single female and later integrated more successfully into unfamiliar mixed-sex groups (Ruploh et al., 2013, 2014). Together, these examples demonstrate that it is essential for adolescent individuals to acquire knowledge of social rules and behavioural patterns necessary for interacting with conspecifics (e.g., social learning) and thus adjusting to their social environment (Lürzel et al., 2010; Zimmermann, Kaiser, Hennessy, et al., 2017; Zimmermann, Kaiser, & Sachser, 2017).

Such an influence of social environment on behavioural phenotypes has also been discussed within the framework of *social niche conformance*, which has gained prominence in behavioural ecology (M. I. Kaiser et al., 2024; Müller et al., 2020; Trappes et al., 2022). The individualized social niche describes the unit that is shaped by social interactions of a focal individual with conspecifics (M. I. Kaiser et al., 2024; Mutwill et al., 2023). Social niche conformance refers to the process by which individuals adjust to an existing social environment through shaping of their phenotype, which can for example be mediated through underlying endocrine mechanisms (M. I. Kaiser et al., 2024; Lilie et al., 2022; Müller et al., 2020; Trappes et al., 2022). These are prominent during adolescence, as both the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-adrenocortical (HPA) axis undergo substantial maturation (McCormick & Mathews, 2010; Peper et al., 2010; Romeo, 2018). The HPG axis regulates the production of sex steroids such as testosterone in males (Peper et al., 2010), whereas the HPA axis regulates the secretion of glucocorticoids (Jacobson & Sapolsky, 1991; Sapolsky, 2002). Glucocorticoids are released to maintain metabolism and homeostasis and increase when energetic demands rise, for example in response to unpredictable challenges. For social animals, this also includes challenges arising from the social environment (Rystrom, Wesseler, et al., 2024). This stressor-induced HPA function, i.e., HPA reactivity, primarily functions to mobilise energy and modulate different behaviours (Koolhaas et al., 1999, 2011; Mikics et al., 2004; Sapolsky, 2002; Sapolsky et al., 2000). Furthermore, HPA reactivity can differ both in its speed, reflecting how rapidly glucocorticoid concentrations change following a challenge, and in its magnitude, reflecting the extent of the increase above baseline (Taff et al., 2022). Interactions between the HPG and HPA axes have been shown to shape behavioural phenotypes and are modulated by the social environment (Mbiydzennyuy & Qulu, 2024; Terburg et al., 2009). Previous studies in juvenile, adolescent and adult male guinea pigs have shown that the social environment can shape testosterone concentrations and HPA reactivity (Gleske et al., 2026; Lürzel et al., 2010, 2011; Mutwill et al., 2020). The distinct behavioural and reproductive

strategies described in adolescent guinea pigs above are also associated with different endocrine phenotypes. The males raised in high population density during adolescence frequently engage in diverse social interactions (Lürzel et al., 2010; Sachser et al., 2013, 2018). These interactions- especially courting and aggressive encounters- trigger increased testosterone levels in male vertebrates (Hirschenhauser & Oliveira, 2006; Lürzel et al., 2010; Sachser et al., 2013). Testosterone in turn has inhibiting effects on the HPA axis (Seale, Wood, Atkinson, Harbuz, et al., 2004), thereby reducing HPA reactivity (Sachser et al., 2013, 2018). In contrast, males housed with only a same-age female during adolescence, i.e., low population density, experience fewer social interactions, leading to lower testosterone levels and higher HPA reactivity (Sachser et al., 2013, 2018). Also in rats (*Rattus norvegicus* f. *domestica*), social status within a group has been suggested to influence adolescent testosterone levels, which may contribute to variation in glucocorticoid receptor expression and the organization of HPA axis regulation (Evuarherhe et al., 2009). Together, these findings illustrate how endocrine adjustments may contribute to social niche conformance during adolescence.

Since adolescence is a highly dynamic and plastic developmental phase characterised by ongoing endocrine, neural and behavioural maturation (Poggi et al., 2026; Romeo et al., 2002; Schneider, 2013; Smith et al., 1996), it is important to investigate how such adjustments unfold over the course of this phase. Although many studies have investigated effects of the social environment on endocrine and behavioural phenotypes in adolescent guinea pigs (Lürzel et al., 2010, 2011; Rystrom, Richter, et al., 2024; Rystrom, Wesseler, et al., 2024; Zimmermann, Kaiser, Hennessy, et al., 2017; Zimmermann, Kaiser, & Sachser, 2017), these studies have typically assessed responses before and after a given social experience. As a consequence, they provide only limited insight into the temporal dynamics of these adjustments. Moreover, social environments can also influence the temporal stability of inter-individual differences in trait expression, i.e., whether individuals consistently differ from one another across repeated measurements (Jäger et al., 2019; Monestier & Bell, 2020; Mutwill et al., 2023). Repeated assessments allow this stability to be quantified as repeatability, i.e., the proportion of total phenotypic variance attributable to between-individual differences (Nakagawa & Schielzeth, 2010; Wolak et al., 2012). Previous work in guinea pigs has shown that social complexity can affect the repeatability of endocrine traits, which was discussed as potentially reflecting the differentiation of individualized social niches (Mutwill et al., 2023). Occupying different social niches within a social group is assumed to avoid social conflict and reduce unpredictability in social encounters (Bergmüller & Taborsky, 2010; Mutwill et al., 2023). Thus, examining repeatability provides a complementary perspective on how social environments shape (endocrine) phenotypes.

The present study therefore aimed to examine effects of different social environments on the endocrine and behavioural phenotype across adolescence in male guinea pigs. All males lived in heterosexual pairs, but males in one condition received additional social stimulation via regular encounters with unfamiliar

animals of both sexes (for details, see methods). This additional social stimulation was intended to create a socially more variable and less predictable environment, characterised by a higher frequency and diversity of social interactions. By repeatedly assessing endocrine and behavioural parameters during adolescence, we examined how the effects of the social environment on these phenotypes unfold over time. Regarding endocrine phenotypes, we assessed testosterone concentrations as well as basal HPA-axis activity and stressor-induced HPA reactivity. To characterise these different components of HPA-axis function, cortisol concentrations, the main glucocorticoid in guinea pigs (Fujieda et al., 1982), were measured immediately before the onset of a novel environment challenge and 1 and 2 h thereafter. The pre-challenge concentration at 0 h was used as an index of baseline HPA-axis activity. The increase in cortisol after 1 h was interpreted primarily as an index of the early speed of the response, whereas the increase after 2 h was interpreted primarily as an index of response magnitude. We also estimated repeatability of all endocrine parameters. Based on the previous findings outlined above, we hypothesised that baseline cortisol, baseline testosterone and cortisol responsiveness as well as their repeatability would differ between males from the two social conditions. Concerning behavioural phenotype, we focused on sociopositive, courtship and sexual behaviour, as these are commonly known to be influenced by social environment during adolescence (Sachser et al., 2020; Zimmermann, Kaiser, Hennessy, et al., 2017; Zimmermann, Kaiser, & Sachser, 2017). These behaviours were assessed in the home enclosure towards the female housing partner and towards unfamiliar adult females and infant males in established behavioural tests conducted in a separate test enclosure, allowing us to compare behavioural responses of males from the two social conditions across different social contexts. To extend behavioural assessment beyond social contexts, we additionally assessed risk-taking behaviour in another behavioural test, as adolescence is often characterised by increased risk-taking behaviour (Laviola et al., 2003; Macrì et al., 2002) which can also be influenced by the social environment (Mudra Rakshasa & Tong, 2020; Potrebic et al., 2021). We hypothesised that behavioural measures would differ between males from the two social conditions. We had no a priori expectation regarding how endocrine and behavioural differences would unfold over time.

2. Material and methods

2.1 Animals and housing conditions

All animals included in this study were bred from a breeding program of multi-coloured shorthaired guinea pigs (*Cavia aperea* f. *porcellus*) at the Department of Behavioural Biology at the University of Münster. They were born and reared in a total of eight harem groups within one breeding room, each consisting of one male, one to three females and their pre-weaned offspring. The offspring was routinely taken out of the harems after weaning at post-natal day (PND) 21 (± 1) and adults were removed and replaced at around 18-24 months of age. Each harem was kept in wooden enclosures on the ground

with a base area of approximately 1.5 m² and a wall height of 0.5 m. The enclosures were filled with wood shavings (Tierwohl Super, J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany) as bedding and enriched with red plastic shelters and wooden bridges.

After weaning, the experimental animals were transferred to enclosures in a different housing room. These enclosures had a base area of 0.5 m², a wall height of 0.5 m, were also filled with wood shavings and enriched with a big and a small red plastic shelter. Food (hasfit Cavia C pellets, EQUOVIS GmbH, Münster, Germany) and water were available *ad libitum*. Since guinea pigs are incapable of synthesizing ascorbic acid (Chatterjee, 1973; Nandi et al., 1997) and therefore prone to vitamin C deficiency (S. Kaiser et al., 2024), a vitamin C supplement (100% L-ascorbic acid, altapharma, Dirk Rossmann GmbH, Burgwedel, Germany) was added to the water three times per week (approximately 120 mg vitamin C in 900 ml water shared between two animals). Additionally, hay was replenished daily. All guinea pig housing rooms were kept under controlled conditions with a 12 h: 12 h light/ dark cycle (lights on at 07:00), temperature of approximately 22 °C and relative humidity of approximately 50 %.

2.2 Experimental design

For this study, twenty male guinea pigs were used. To investigate the influence of distinct social environments on hormonal and behavioural phenotypes, they were randomly assigned to one of two social conditions. All males lived in heterosexual pairs during adolescence, however, males of one group were additionally socially stimulated (see 2.3) regularly (pair-housed male with additionally social stimulation; PM+S condition), whereas males of the other group were not (pair-housed male without additional social stimulation; PM-S condition). The twenty males were organized into ten matched pairs, each consisting of one PM+S male and one PM-S male.

After weaning at PND 21 (± 5), the two males within one matched pair were initially housed together. Their future female partners, which were about the same age, were housed together in an adjacent enclosure. Each male and his respective female partner stem from different harem groups, ensuring they were neither half nor full siblings. These pre-experimental housing conditions were maintained until the focus males reached early adolescence at PND 53 (± 5). At that time, the two males were separated and each one was housed with his assigned female partner.

Following a three-day habituation period, the experiments started at PND 56 (± 5) and lasted seven weeks, meaning the animals were 102 (± 5) days of age when the experiments ended. In guinea pig males, adolescence spans approximately from PND 55 to PND 120 (Lürzel et al., 2010). Sexual maturity is usually reached around PND 70 (Trillmich et al., 2006), but marks the completion of puberty rather than the end of adolescence, which encompasses the broader transition from the juvenile to the adult phenotype (Bell, 2018; Spear, 2000). Consistent with this distinction, males do not reach their full body

size until approximately 8-12 months of age and become socially mature enough to attain dominant positions only at around seven months, indicating that important aspects of somatic and social development continue beyond sexual maturity (S. Kaiser et al., 2024; Sachser, 1986). The home enclosures of both males within a matched pair, each now housed together with their respective female partner, were placed side by side in the same housing room. This was done to control for variability in housing conditions such as room temperature or humidity. All experimental procedures (cortisol response tests, body weight measurements, video recordings, behavioural tests) were conducted in parallel within each matched pair to minimise variability in timing of the experiments.

In total, four cortisol response tests (CRTs) to measure basal and reaction cortisol values (*see assessment of endocrine phenotypes*) were conducted within the seven week long experimental phase (**Fig. 1**). The first CRT was conducted before the social stimulation treatment started and is thus referred to as CRT0. CRT0 was conducted in the first experimental week three days after the focus males were housed together with their female partners. CRT1, CRT2 and CRT3 followed 14 (± 2) days after the preceding one, with CRT1 being conducted approximately around the onset of sexual maturity (**Fig. 1**). To additionally assess behavioural changes over time, home enclosure behaviour was video-recorded from the second experimental week onwards. The six weeks of behavioural observations were divided into three consecutive two-week phases (phase 1 - phase 3), matching the timing of CRT1, CRT2 and CRT3 (**Fig. 1**). The social stimulation treatment started in the first experimental week after CRT0 and continued throughout the whole experiments. The days and times of social stimulation sessions and home-enclosure recording were randomised separately across the week to avoid possible habituation effects and to observe behaviour at different times of day. In the final experimental week, an additional battery of behavioural tests was conducted to further evaluate social and risk-taking behaviour. These tests comprised the step-down test (SDT), the male-female interaction test (MFIT) and the social initiative test (SIT) (*see 2.5.2*). In addition, as part of another project, fur swabbing with PDMS (Polydimethylsiloxane) tubes to analyse chemical fingerprints was conducted. The procedural order of CRT3, behavioural tests and fur swabbing in the last experimental week varied across individuals, but was identical within each matched pair. The focus males never experienced more than one of these procedures per day.

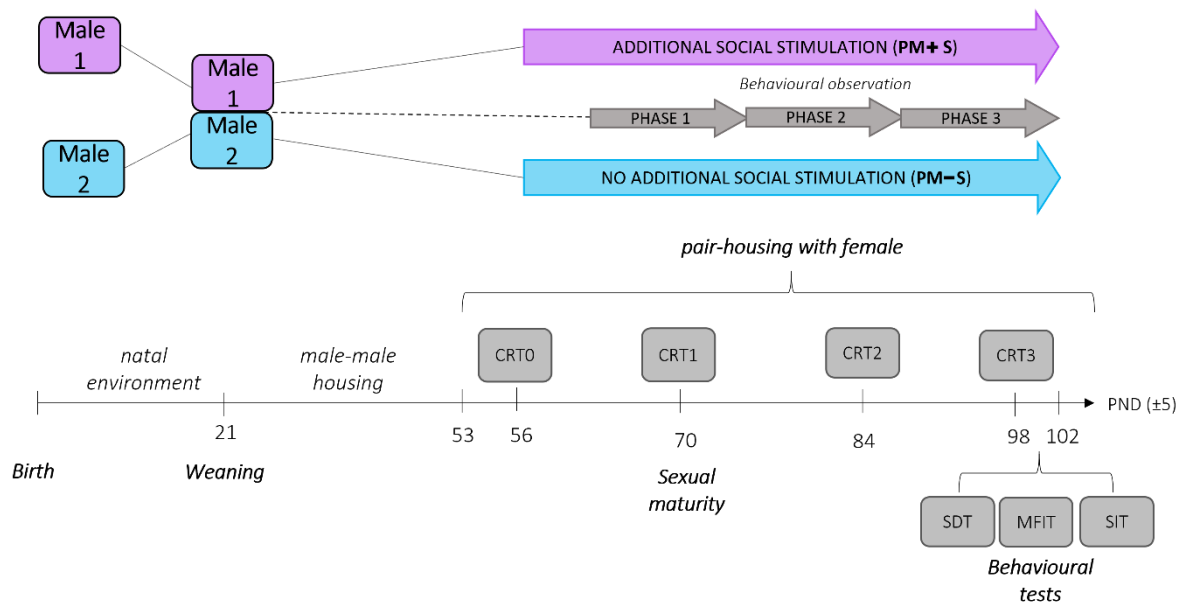


Figure 1: Experimental design. After weaning, two males, subsequently assigned to the PM+S (pair-housed males with additional social stimulation) and PM-S (pair-housed males without additional social stimulation) conditions, were housed together. Colours indicate the males' subsequent treatment allocation but do not represent treatment exposure before the experimental phase. At post-natal day (PND) 53 ± 5 , the males were separated and each was pair-housed with an assigned female partner. Following a three-day habituation period, the experimental phase began at PND 56 ± 5 with the first cortisol response test (CRT0). After CRT0, males in the PM+S condition received additional social stimulation through regular encounters with unfamiliar conspecifics, whereas males in the PM-S condition received no additional social stimulation. In total, four cortisol response tests (CRT0-CRT3) were conducted to assess endocrine phenotypes. Home-enclosure behaviour was recorded from the second experimental week onwards and grouped into three phases of two weeks each (Phases 1-3). In the final experimental week, three additional behavioural tests were conducted: the step-down test (SDT), male-female interaction test (MFIT) and social initiative test (SIT). The experimental phase ended at PND 102 ± 5 . Developmental milestones are indicated along the timeline: birth (PND 0), weaning (PND 21) and sexual maturity (around PND 70).

2.3 Social stimulation

The social stimulation procedure applied in the present study was adapted from earlier studies, where additional social stimulation successfully influenced hormonal profiles in juvenile and adolescent guinea pig males (Gleske et al., 2026; Lürzel et al., 2010, 2011). The social stimulation for the respective males (PM+S) started after CRT0 and was applied three times per week for the whole experimental phase of seven weeks. Each social stimulation session consisted of introducing an unfamiliar conspecific into the home enclosure of the focus male and his female partner for up to ten minutes. Per week, two social stimulation sessions were done with another male and one with a female, resulting in a total of 14 male and seven female stimulation sessions per focus male. The female stimulation animals always came from the harems to ensure they were pregnant and thus in the same reproductive stadium, preventing a confounding influence of oestrus. In total, 30 stimulus animals were used (14 males, 16 females). If the focus male was stimulated more than once with the same stimulus animal, there was always a minimum interval of seven days between these stimulation sessions.

PM+S males never experienced more than one social stimulation session per day. To reduce potential habituation effects, both the day of the week and time of day of stimulation sessions were varied. Prior to each session, the red plastic shelters were temporarily removed from the home enclosure of the focus male and the video camera was turned on, as all stimulation sessions were recorded. After the stimulation animals were introduced into the home enclosure, a timer was started as the sessions had maximum length of ten minutes. Sessions were terminated earlier if aggressive behaviour escalated in order to minimise the risk of injury. Out of a total of 140 stimulation sessions using male stimulus animals, 21 were aborted due to escalating aggression.

2.4 Assessment of hormone concentrations

Hormones were measured using blood samples obtained in cortisol response tests (CRTs), a standardized test in which the endocrine stress response was assessed at different time points following exposure to a novel environment as stressor (Gleske et al., 2026; Hennessy et al., 2006; Rystrom et al., 2022). The test started between 12:30 and 13:30, as plasma cortisol concentrations fluctuate throughout the day and a peak is observed at 13:00 (Sachser, 1994). Prior to that, the animals were undisturbed for one hour.

At the start of the CRT, the male was taken out of his home enclosure and placed on the experimenter's lap outside of the housing room. To facilitate blood flow, an ointment (Finalgon® Wärmesalbe DUO, Zentiva Pharma GmbH, Frankfurt am Main, Germany) for expanding the blood vessels was applied to the guinea pig's ear and wiped off again. After that, the marginal ear vessel was punctured with a lancet (Solofix® Blutlanzetten, B. Braun Melsungen AG, Melsungen, Germany) and blood was collected in heparinized capillary tubes (Capillary tubes for microhaematocrits, 100 µl, Paul Marienfeld GmbH & Co KG, Lauda Königshofen, Germany) to later on determine basal cortisol (c0) and basal testosterone (t) levels. For cortisol, this procedure had to be completed within 3 minutes to prevent the sampling process itself from influencing the hormone values in the obtained sample itself (Sachser, 1994). Such stress-induced changes in hormone values appear later in testosterone than in cortisol (Sachser, 1994) and collecting a sufficient amount of blood for testosterone assays requires slightly more time. Therefore, blood samples for testosterone were collected within 6 minutes, which represents a compromise between sample quality and animal welfare and follows the procedure applied in earlier studies (e.g., (Gleske et al., 2026; Mutwill et al., 2020, 2021; Zimmermann, Kaiser, Hennessy, et al., 2017; Zimmermann, Kaiser, & Sachser, 2017)). Then, the guinea pig was singly placed into an unfamiliar enclosure in a different housing room where it stayed for a total of two hours. This enclosure had a size of 1 m², wall height of 0.5 m and was equipped with wood shavings, food and water. Exactly one and two hours after the first one, blood sampling was repeated to determine first (c1) and second (c2) cortisol responsiveness. These measurements represented HPA reactivity to the standardized CRT

procedure as a whole, including handling, separation from the female partner and exposure to the unfamiliar enclosure. The guinea pigs were weighed after each blood sampling and returned to their home enclosure after the last one.

To separate the blood plasma, the sample was centrifugated ($13,000 \times g$ for 5 min), transferred into a 1.5 mL Eppendorf tube and deep frozen at -20°C until assayed. Hormone concentrations were determined in duplicate using enzyme-linked immunosorbent assays (ELISA) (cortisol: RE52061, IBL International, Hamburg, Germany; antibody cross-reactivity: cortisol (100%), prednisolone (30%), 11-deoxycortisol (20%), cortisone (10.7%), prednisone (6.5%), 17 α -hydroxyprogesterone (5.4%), 6 β -hydroxycortisol (4.4%), corticosterone 3.8%, desoxycorticosterone (1.8%); testosterone: RE52151, IBL International, Hamburg, Germany; antibody cross-reactivity: testosterone 100%, 11 β -OH-testosterone 8.7%, 11 α OH-testosterone, 3.2%, dihydrotestosterone 1.9%). Intra- and inter-assay CVs were quantified as 2.09% and 3.98% for cortisol and 4.7% and 5.7% for testosterone.

In some cases, it was not possible to obtain a sufficient amount of blood for the ELISA within the predefined sampling time limits. Blood collection was not continued beyond these limits to avoid compromising sample validity and animal welfare. For each CRT, the sample size per group ranged between $n = 4$ and $n = 10$. Detailed sample sizes for each hormone measurement and CRT are provided in the supplementary material (**Tab. S4**)

2.5 Assessment of behavioural parameters

2.5.1 Home enclosure behaviour

To examine how distinct social environments influence social behaviour, home enclosure behaviour of the focus males in both conditions was recorded (outside of social stimulation sessions for PM+S males). Video recordings were conducted twice per week for one hour each, starting from the second experimental week onwards when PM+S males already experienced some social stimulation sessions. For this purpose, a video camera (Panasonic HC-V785 or SONY HDR-CX405) was installed approximately 1.5 m above each experimental animal's home enclosure. The day and time (between 08:00 and 17:00) at which the videos were recorded was randomized. In total, 12 h of home enclosure behaviour was collected for each individual. Video analysis was done by a human observer with the program Interact (Interact, Lab Suite Version 2022, Program version 20.8.3.0, Mangold International GmbH, Arnstorf, Germany). The videos were blinded and randomized, ensuring ID and treatment of the respective individual as well as the time of recording were unknown to the observer. Continuous focal animal sampling was used (Altmann, 1974). The entire hour from each video recording was reviewed and all visible occurrences of the predefined behaviours shown by the focal male were coded as events. These included behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal

sniffing), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt). As agonistic behaviour rarely occurs between male and female guinea pigs (Sachser et al., 1994; Sachser & Lick, 1989, 1991), it was not included in the ethogram. The full ethogram can be found in the supplementary material (**Tab. S1**). If no occurrence of a behavioural category was recorded during a video, that category was assigned a value of zero.

2.5.2 Behavioural tests

A battery of behavioural tests was performed across three days during the seventh (final) experimental week, when males were approximately at PND 100 ± 5 . These tests included the step-down test (SDT), male-female interaction test (MFIT) and social initiative test (SIT). They were adapted from previous studies implementing these tests in guinea pigs and wild cavies (Zipser et al., 2013, 2014). Each behavioural test was conducted on a separate day. On a given test day, both males within a matched pair were tested consecutively in that same test, with the order of testing (PM+S first vs. PM-S first) randomized. Similar to the CRT, all behavioural tests were conducted in an unfamiliar enclosure in a different housing room. This test enclosure had a size of 1 m^2 , wall height of 0.5 m and was equipped with wood shavings. For the SDT, the experimenter stayed in the room, whereas the MFIT and SIT were videotaped and conducted without the experimenter being present. For this purpose, a video camera (Panasonic HC-V785 or SONY HDR-CX405) was installed approximately 1.5 m above the test enclosure. Before a test started, the focus male was taken out of his home enclosure and carried to the test enclosure in a Makrolon type II cage. The individual tests are described in more detail below.

2.5.2.1 Step-down test (SDT)

The SDT is a test to assess risk-taking behaviour (Zipser et al., 2013, 2014). An elevated, sheltered, square platform (900 cm^2 base area; 23 cm height) was placed in the centre of the test enclosure. The platform was covered with wood shavings. The focus male was removed from the transport cage and placed on the platform facing away from the experimenter. The focus male was gently held still until his reflex to escape the experimenter subsided. The experimenter then stepped back and measured the focus male's latency to step down from the platform. This was defined as the time point at which the animal touched the floor of the enclosure with all four paws. A maximum test duration of 15 minutes was applied. If the focus male did not step down within this time frame, the test was terminated and latency was recorded as 15 minutes (900 seconds).

2.5.2.2 Male-female interaction test (MFIT)

The MFIT was performed to assess how males with and without additional social stimulation interact with an unfamiliar female conspecific. Similar to social stimulation, the females used for the MFIT stem from the harem groups and were therefore not in oestrus during the tests. Within each matched pair,

the same female was used. Prior to the test, the test enclosure was divided into two equal halves by a mesh partition. The female was placed in one half of the enclosure and the focus male in the other. The experimenter stepped back and the animals had an acclimation period of 60 seconds to initiate first visual and olfactory contact through the mesh. Afterward, the partition was removed and the animals could freely interact for 30 minutes. During this time, the experimenter left the room. The entire session was recorded and videos were analysed with the program Interact (Interact, Lab Suite Version 2022, Program version 20.8.3.0, Mangold International GmbH, Arnstorf, Germany). The videos were blinded and randomized, ensuring ID and treatment of the respective individual were unknown to the observer. The analysis started after the acclimation period was over and the mesh removed. Latency until first contact with female and behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing, time spent near female), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt) were analysed (**Tab. S2**). Due to technical issues with the camera, one MFIT video was missing in the PM-S group; thereby reducing the sample size to $n = 9$ in that group.

2.5.2.3 Social initiative test (SIT)

The SIT was conducted to investigate general motivation of the focus males to initiate social contact (Zipser et al., 2014). For this purpose, an unfamiliar male infant (5 to 15 days of age) was used as interaction partner to minimize the likelihood that the behaviour of the focus males was driven by either sexual or agonistic motivation (Zipser et al., 2014). These infants had not yet been weaned, meaning they were still living with their mothers in their natal groups. Within each matched pair, the same male infant was used for both consecutive tests and was returned to its mother and natal group after completion of both tests. Prior to the test, the infant was placed into the test enclosure under a small grated metal basket (25 x 21 x 15 cm) turned upside down to prevent approaches initiated by the infant. As control, an identical but empty basket was also placed into the enclosure. Both baskets were positioned in the centre of the enclosure, approximately 20 cm apart and about 20 cm from the left or right wall, respectively. The focus male was then placed into the enclosure facing away from the experimenter and towards the baskets. The experimenter left the room and the focus male was allowed to make contact to the infant for 15 minutes (basket phase). After this time, the experimenter quietly re-entered the room, removed both baskets and the animals were allowed to freely interact for an additional 15 minutes (no basket phase), during which the experimenter again left room. The entire session was recorded and videos were analysed with the program Interact (Interact, Lab Suite Version 2022, Program version 20.8.3.0, Mangold International GmbH, Arnstorf, Germany). The videos were blinded and randomized, ensuring ID and treatment of the respective individual were unknown to the observer. The analysis was divided into basket phase and no basket phase. During the no basket phase,

the following parameters were analysed: frequency of empty basket sniffing, frequency of infant basket sniffing, time spent near empty basket and time spent near infant basket. For the no basket phase, latency until first contact with infant and behaviours from the categories sociopositive behaviour (nasal sniffing, naso-anal sniffing, time spent near infant), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt) were analysed (**Tab. S3**). Due to technical issues with the camera, one SIT video was missing in the PM+S group; thereby reducing the sample size to $n = 9$ in that group.

2.6 Statistics

Data analysis was carried out with RStudio version 2022.07.0 (R Core Team, 2022). The total sample size was $n = 20$ animals, with $n = 10$ animals per social condition. Descriptive statistics, model summaries and detailed test statistics for all analyses can be found in the supplementary material.

For the mixed-effects models used to analyse hormone concentrations and home enclosure behaviour, the fixed- and random-effect structures were selected via likelihood ratio tests (LRTs) based on maximum-likelihood estimation (see supplementary material, **Tab. S10+S20**). In a first step, a full model containing the interaction between social condition and time (CRT or phase) was compared with a reduced model containing only the corresponding main effects. The interaction was retained only when its addition significantly improved the goodness of fit. In a second step, the random-effect structure was evaluated. Since the males were organised into matched pairs, the models selected in the first step were subsequently fitted either with “individual ID” as the only random effect or with “individual ID” nested within “pair ID”. Models accounting for “pair ID” frequently resulted in singular fits, while including pair ID also did not significantly improve model fit for any of the hormone or home enclosure behaviour variables. Therefore, pair ID was not included in the final statistical analyses.

2.6.1 Hormone concentrations

Linear mixed-effects models using Gaussian error distributions were used to analyse the influence of the social condition on hormone concentrations using the *lme4* (Bates et al., 2015) and *lmerTest* package (Kuznetsova et al., 2017). In total, four models were fitted with 1) baseline cortisol, 2) baseline testosterone, 3) increase in cortisol responsiveness after 1 hour and 4) increase in cortisol responsiveness after 2 hours as a respective response variable. Increase in cortisol responsiveness after 1 hour and after 2 hours was calculated by subtracting baseline cortisol values from the absolute values of cortisol responsiveness after 1 hour and after 2 hours. This was done to eliminate confounding effects caused by different cortisol baseline values. Social condition (additional social stimulation versus no additional stimulation), time (CRT) and their interaction were initially included as fixed effects to investigate whether changes in hormone concentrations over time differed between the two social

conditions. Based on the model-selection procedure described above, the interaction between social condition and CRT was retained in the final model for baseline cortisol. For baseline testosterone and cortisol responsiveness after 1 and 2 hours, the interaction did not significantly improve model fit (see supplementary material, **Tab. S10**). Therefore, the final models for these variables included only the main effects of social condition and CRT. CRT represented the first, second and third CRT conducted after treatment (social stimulation) started. As the focus of this study was to investigate the effects of additional social stimulation on hormone concentrations over time, data from CRT0 was excluded from the models as it was conducted before the social stimulation treatment started. However, hormone concentrations at CRT0 were still compared between the treatment groups using independent-sample Wilcoxon rank-sum tests to confirm there were indeed no differences between the groups prior to treatment. Furthermore, the continuous variable body weight was first mean-centered and then included as a fixed effect because earlier studies in guinea pigs have shown that body weight can influence hormone concentrations (Mutwill et al., 2023; Rystrom, Wesseler, et al., 2024). For each CRT, the first body weight measurement obtained during the respective CRT was used. “Individual ID” was included as a random effect in all final models. We used the *performance* (Lüdecke et al., 2021) and *DHARMA* package (Hartig, Florian, 2022) to check model assumptions. Marginal and conditional R^2 values were calculated using the *performance* package (Lüdecke et al., 2021), while partial R^2 values for individual predictors were calculated using the *sensmakr* package (Cinelli et al., 2024). Pairwise comparisons for treatment, CRT and treatment*CRT interaction were done by applying Tukey’s adjustment for multiple comparison using the *emmeans* package (Lenth, 2024).

Adjusted repeatability estimates of hormone concentrations were calculated for each of the treatment groups using the *rptR* package (Stoffel et al., 2017). 95% confidence intervals were determined by parametric bootstrapping (N = 1000), and likelihood ratio tests were used for significance testing. The models used to estimate adjusted repeatability were the same as mentioned before, with the only exception that treatment was removed as fixed effect.

2.6.2 Behaviour

2.6.2.1 Home enclosure behaviour

For the analysis of the home enclosure behaviour, behavioural counts from the coded videos were aggregated for each individual and phase by summing all observations within the respective phase. Phase 1 comprised the first and second week of video recordings, phase 2 the third and fourth week and phase 3 the fifth and sixth week. Behaviour was pooled into the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing) and courtship behaviour (ano-genital licking, rumba) with individual behaviours being summed within each category. Behaviour from the category sexual

behaviour (mating and mating attempt) was only observed in 4 out of 240 videos in total and therefore excluded from statistical analysis.

Sociopositive and courtship behaviour were analysed using generalized linear mixed-effects models with negative binomial distribution and quadratic parameterisation, since the count data was strongly skewed. Models were fitted using the *glmmTMB* package (Brooks et al., 2017). Similar to the hormone concentration analysis, social condition, time (phase) and their interaction were initially included as fixed effects. However, the interaction between social condition and phase did not significantly improve model fit for either sociopositive or courtship behaviour (see supplementary material, **Tab. S20**). Therefore, the final models included only social condition and phase as main effects. ID was again fitted as a random effect. Model assumptions as well as the estimation of the different R^2 values were conducted in the same manner as for the analysis of hormone concentrations. Models were additionally checked for overdispersion and zero-inflation using the *performance* package (Lüdtke et al., 2021). Pairwise comparisons for treatment and phase were done by applying Tukey's adjustment for multiple comparison using the *emmeans* package (Lenth, 2024).

2.6.2.2 Behavioural tests

Prior to statistical analyses, normality of the data was assessed using Shapiro-Wilk tests. Depending on the distribution of the data, either parametric (Welch's t-test) or non-parametric tests (independent-sample Wilcoxon rank-sum tests) were used.

Data of the step-down test (SDT) was analysed in two steps. First, the proportion of animals stepping down from the platform within the maximum test duration was compared between the two social conditions. For this purpose, a binary variable was created indicating whether an individual stepped down during the test (latency < 900 s) or not (latency = 900 s). Differences between social conditions were analysed using Fisher's exact test. In a second step, the latency to step down from the platform was analysed only for individuals that stepped down during the test (latency < 900 s). Differences in latency between the two social conditions were assessed using an independent-sample Wilcoxon rank-sum test.

Data from the male-female interaction test (MFIT) was analysed by comparing multiple behavioural measures between the two social conditions. These variables included latency to first contact with the female, time spent near the female as well as behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing), courtship behaviour (ano-genital licking, rumba) and sexual behaviour (mating and mating attempt). For each individual, behaviours belonging to the same category were summed before analysis. Each variable was analysed using either Welch's t-tests (time spent near

female, sociopositive behaviour, courtship behaviour) or Wilcoxon rank-sum tests (latency to first contact with female, sexual behaviour).

Data from the social initiative test (SIT) was analysed separately for the “basket phase” and the “no basket phase”. For the “basket phase”, the influence of social condition and basket type (empty vs. infant) on the number of sniffs and the duration of time spent near the baskets was analysed. Sniffing behaviour, as the total count of sniffs, was analysed using a generalized linear mixed-effects models with Poisson distribution, whereas the duration of time spent near the baskets was analysed using a linear mixed-effects model. To improve variance homogeneity of model residuals, duration data was log-transformed prior to analysis. Models were fitted using the *lme4* (Bates et al., 2015) and *lmerTest* package (Kuznetsova et al., 2017). Treatment, basket type and their interaction were included as fixed effects, while ID was fitted as a random effect. We used the *performance* (Lüdtke et al., 2021) and *DHARMa* package (Hartig, Florian, 2022) to check model assumptions. Marginal and conditional R^2 values were calculated using the *performance* package (Lüdtke et al., 2021), while partial R^2 values for individual predictors were calculated using the *sensmakr* package (Cinelli et al., 2024). Pairwise comparisons for treatment, basket type and treatment*basket type interaction were done by applying Tukey’s adjustment for multiple comparison using the *emmeans* package (Lenth, 2024). For the “no basket phase”, the analysed variables included latency to first contact with the infant, time spent near the infant as well as behaviours from the categories sociopositive behaviour (naso-nasal sniffing, naso-anal sniffing, time spent near infant) and courtship behaviour (ano-genital licking, rumba). Sexual behaviour (mating and mating attempt) was excluded from statistical analysis as it occurred only in 5 out of 19 individuals and at very low frequencies. Within each behavioural category, individual behaviours were summed prior to analysis. Each variable was analysed using either Welch’s t-tests (time spent near infant, sociopositive behaviour, courtship behaviour) or independent-sample Wilcoxon rank-sum tests (latency to first contact with infant).

3. Results

3.1 Effects of social environment on hormone concentrations

Wilcoxon rank-sum tests revealed no significant differences in hormone concentrations (c0, c1, c2 and testosterone) between the social conditions at CRT0, prior to the start of treatment (see supplementary material, **Tab. S9**).

Regarding baseline cortisol levels, a significant treatment-by-time interaction effect was found between CRT1 and CRT3 ($\beta = 110.79 \pm 40.31$, $t = 2.75$, $p = 0.012$) (see supplementary material, **Tab. S15**). PM+S males showed a decrease in c0 values from 165.72 ± 65.82 ng/ml at timepoint CRT1, two weeks after the treatment start, to 137.67 ± 46.03 ng/ml at timepoint CRT3, six weeks after the treatment start. In

contrast, PM-S males showed an increase in baseline cortisol levels from 133.48 ± 62.46 ng/ml at timepoint CRT1 to 197.90 ± 80.83 ng/ml at timepoint CRT3 (means $\pm$ SD; see supplementary material, **Tab. S4**) (**Fig. 2a**). This crossover interaction between social condition and CRT for baseline cortisol was statistically supported in comparison to a null model ($\chi^2 = 8.161$, $p = 0.017$) (see supplementary material, **Tab. S10**).

For baseline testosterone levels (**Fig. 2b**), neither a significant effect of treatment nor time (CRT) was found (see supplementary material, **Tab. S18**).

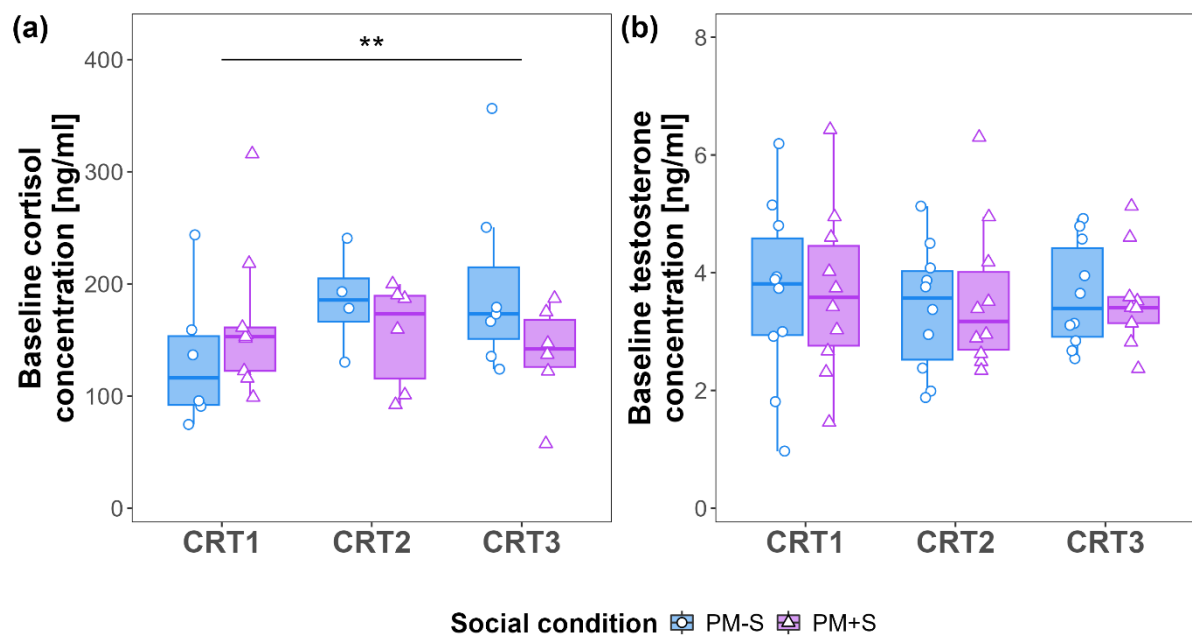


Figure 2: Baseline cortisol (**a**) and testosterone (**b**) concentrations (ng ml⁻¹) two weeks (CRT1), four weeks (CRT2) and six weeks (CRT3) after treatment start. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range. Significance line indicates significant treatment-by-time interaction effect. ** $p < 0.01$.

Regarding increase in cortisol responsiveness after 1 hour (c1) and 2 hours (c2) of exposure to a novel environment, neither a significant effect of treatment nor time (CRT) was found (see supplementary material, **Tab. S16+S17**) (**Fig. 3**).

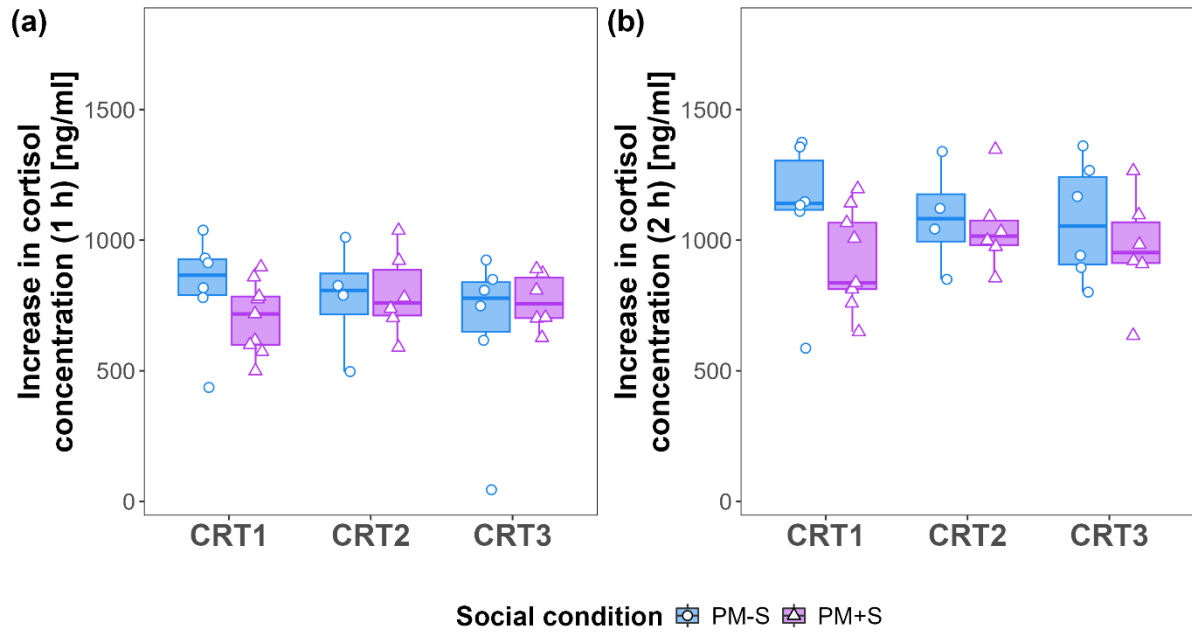


Figure 3: Increase in cortisol concentrations (ng ml^{-1}) at one hour (a) and two hours (b) of exposure to a novel environment two weeks (CRT1), four weeks (CRT2) and six weeks (CRT3) after treatment start. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range.

Adjusted repeatability was analysed for hormone concentrations (baseline cortisol, baseline testosterone, cortisol responsiveness after 1 and 2 hours) in both social conditions (**Fig. 4**). Baseline cortisol (c_0) was not significantly repeatable under any social condition (PM+S group: $R = 0.076$, $CI = [0, 0.685]$, $p = 0.392$; PM-S group: $R = 0.124$, $CI = [0, 0.833]$, $p = 0.401$). Similarly, baseline testosterone was not repeatable in either of the two social conditions (PM+S group: $R = 0.244$, $CI = [0, 0.669]$, $p = 0.153$; PM-S group: $R = 0.346$, $CI = [0, 0.732]$, $p = 0.076$). In contrast, repeatability of increase in cortisol responsiveness after 1 hour (c_1) differed between the two social conditions: while it showed high and significant repeatability in the PM+S group ($R = 0.721$, $CI = [0.233, 0.945]$, $p < 0.001$), it showed very low and non-significant repeatability in the PM-S group ($R = 0.121$, $CI = [0, 0.877]$, $p = 0.432$). For increase in cortisol responsiveness after 2 hours (c_2) we found high and significant repeatability in the PM-S group ($R = 0.782$, $CI = [0.321, 0.980]$, $p = 0.007$). In contrast, repeatability in the PM+S group was moderate but not statistically significant ($R = 0.418$, $CI = [0, 0.864]$, $p = 0.056$).

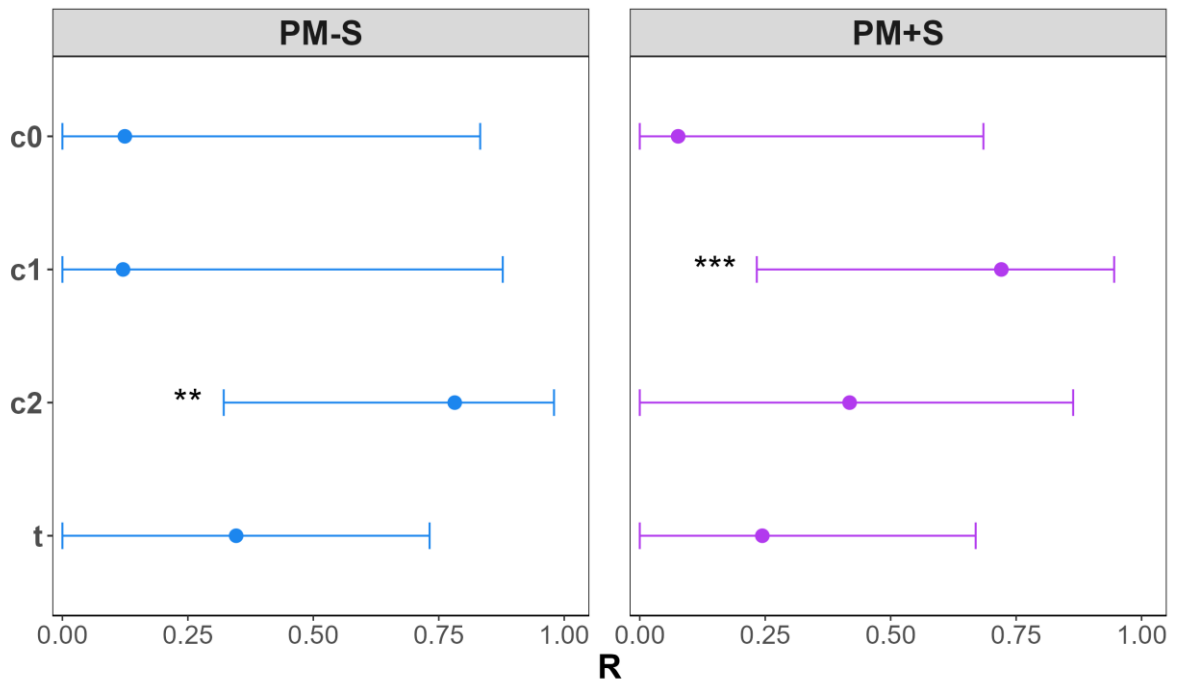


Figure 4: Repeatability (R) of baseline cortisol (c0), increase in cortisol responsiveness after 1 (c1) and 2 hours (c2) of exposure to a novel environment and baseline testosterone (t). Males were either additionally socially stimulated (PM+S) or not (PM-S). Plotted are adjusted repeatability (data points) and confidence intervals (whisker). **p < 0.01, *** p < 0.001.

3.2 Effects of social environment on behaviour

Inclusion of the interaction between social condition and phase did not significantly improve model fit for either sociopositive or courtship behaviour. Therefore, the final models included only the main effects of social condition and phase. For both sociopositive and courtship behaviour, a significant main effect of social condition was found, with PM+S males displaying significantly more sociopositive behaviour ($\beta = -0.566 \pm 0.211$, $z = -2.678$, $p = 0.007$) and courtship behaviour ($\beta = -0.820 \pm 0.371$, $z = -2.209$, $p = 0.027$) than PM-S males (Fig. 5a+5c). Neither for sociopositive behaviour nor courtship behaviour a significant effect of phase was found (see supplementary material, Tab. S23+S24) (Fig. 5b+5d).

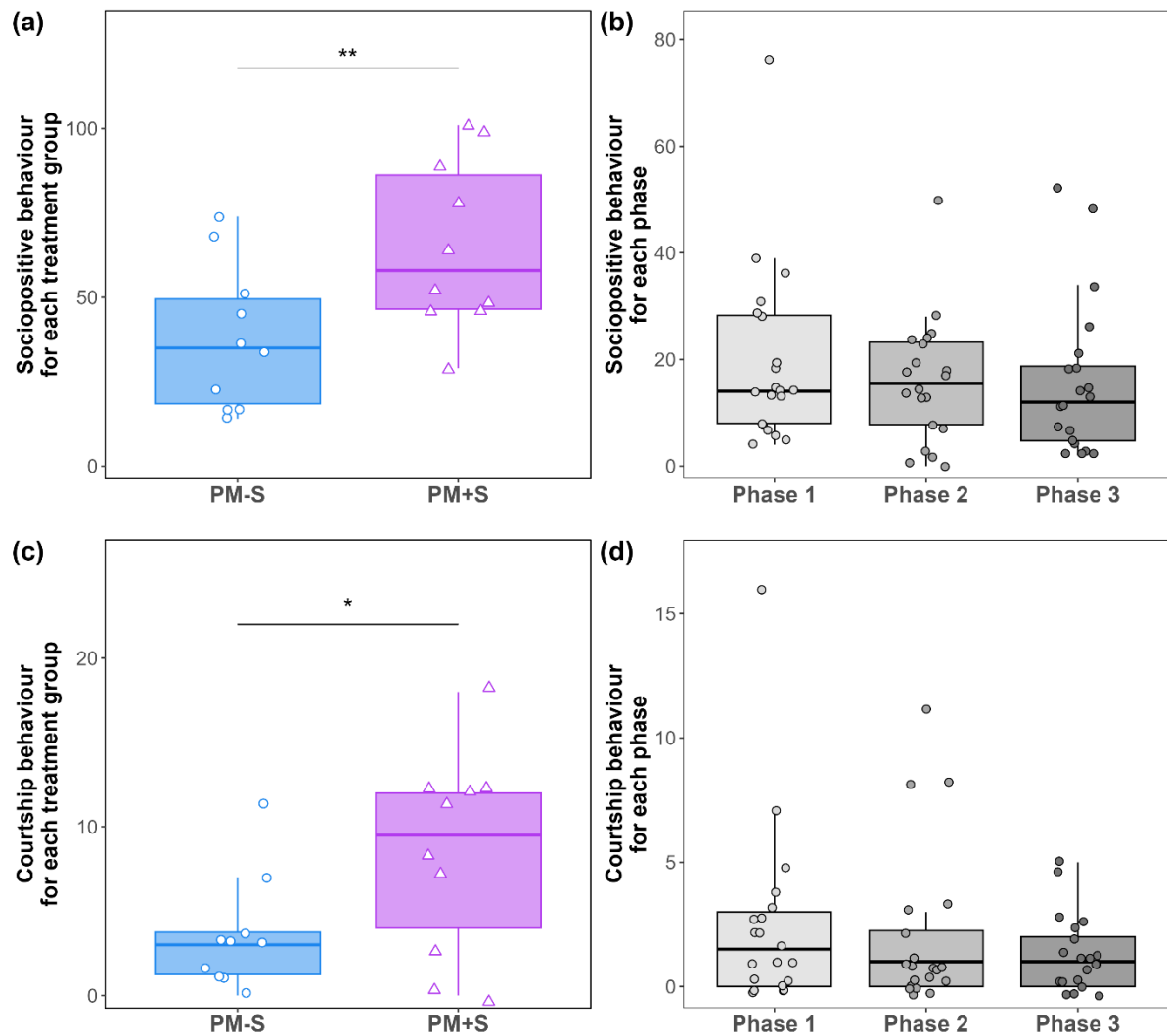


Figure 5: Home enclosure behaviour. Sociopositive behaviour (**a, b**) and courtship behaviour (**c, d**). Behavioural counts are summed across “Phase 1” (1st and 2nd week of video recordings), “Phase 2” (3rd and 4th week of video recordings), and “Phase 3” (5th and 6th week of video recordings) in panels (**a**) and (**c**) and represented separately for all three phases in panels (**b**) and (**d**). Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range. * $p < 0.05$, ** $p < 0.01$.

Data from the step-down test (SDT) was analysed in a similar two-step approach. First, we analysed whether individuals stepped down from the platform within the maximum test duration or not. No significant difference between the two social conditions was found in the proportion of individuals stepping down (see supplementary material, **Tab. S25** and **Fig. S1a**). In a second step, we analysed the latency to step down from the platform for those individuals that stepped down during the test. Also here, no significant difference between the two social conditions was detected (see supplementary material, **Tab. S25** and **Fig. S1b**).

In the male-female interaction test (MFIT), males from the two social conditions did not differ in latency to approach the female or in the time spent near the female (see supplementary material, **Tab. S26** and

Fig. S2a + S2b). Also, for sexual behaviour, no significant difference between social conditions was found (see supplementary material, **Tab. S26** and **Fig. S2C**). Males from the PM+S group exhibited sociopositive and courtship behaviour more often than males from the PM-S group (sociopositive behaviour: $t = -2.43$, $d = 1.09$, $p = 0.029$; courtship behaviour: $t = -2.36$, $d = 1.06$, $p = 0.032$) (**Fig. 6**).

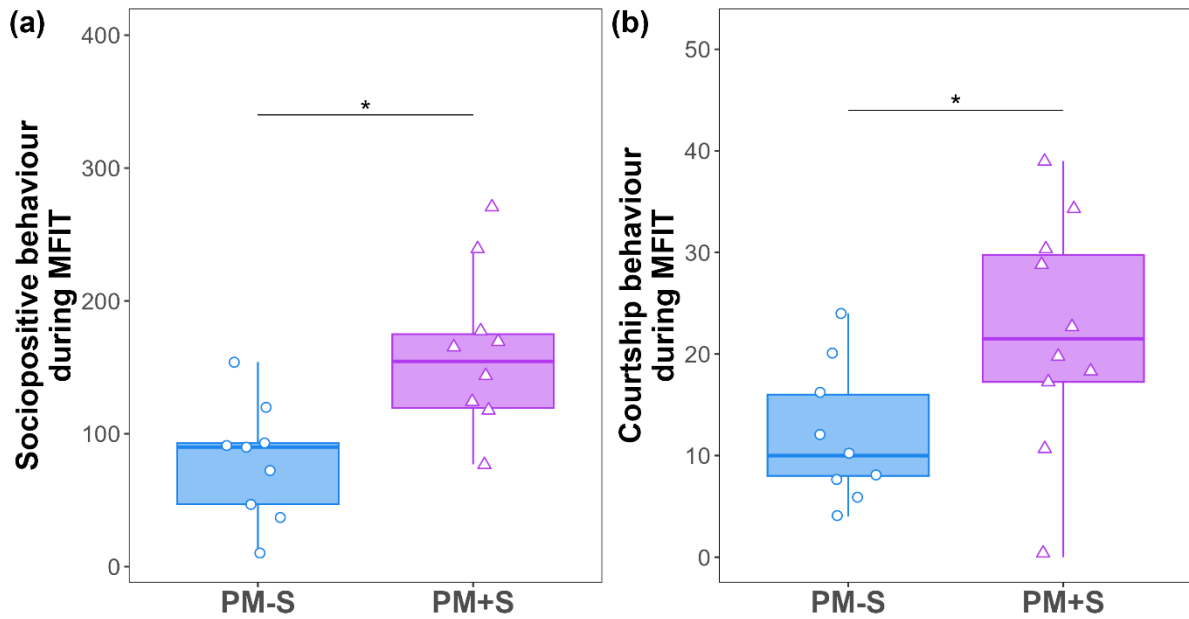


Figure 6: Male-female interaction test (MFIT). Behavioural counts of **(a)** sociopositive behaviour and **(b)** courtship behaviour towards female. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range. * $p < 0.05$.

Data from the social initiative test (SIT) was analysed separately for the “basket phase” and the “no basket phase”. During the “basket phase”, no significant differences between the two social conditions were found for sniffing behaviour or time spent near the baskets (see supplementary material, **Tab. S30+S31**). However, a significant effect of basket type was detected for both variables, with higher sniffing frequencies and more time spent near the infant basket compared to the empty basket in both social conditions (sniffing: PM-S: $\beta = -0.807 \pm 0.090$, $z = -9.005$, $p < 0.001$; PM+S: $\beta = -0.843 \pm 0.101$, $z = -8.345$, $p < 0.001$; time spent near baskets: PM-S: $\beta = -1.32 \pm 0.26$, $t = -5.16$, $p < 0.001$; PM+S: $\beta = -1.47 \pm 0.27$, $t = -5.46$, $p < 0.001$) (see supplementary material, **Tab. S30+S31** and **Fig. S3**). No significant treatment-by-basket interaction effect was found for either variable (see supplementary material, **Tab. S30+S31**). During the “no basket phase”, males from the two social conditions did not differ in latency to approach the infant or time spent near the infant (see supplementary material, **Tab. S27** and **Fig. S4**). However, males from the PM+S group exhibited significantly more sociopositive behaviour than males from the PM-S group ($t = -2.81$, $d = 1.33$, $p = 0.016$) (**Fig. 7a**). Regarding courtship behaviour, no

significant difference between treatment groups was found (see supplementary material, **Tab. S27**) (**Fig. 7b**).

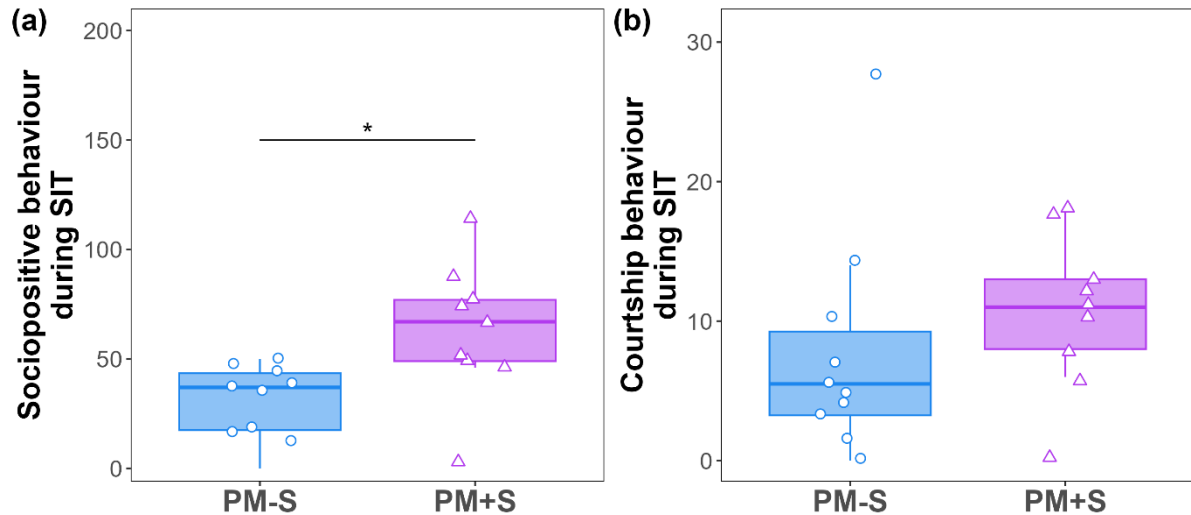


Figure 7: Social initiative test (SIT), no basket phase. Behavioural counts of **(a)** sociopositive behaviour and **(b)** courtship behaviour towards infant. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range. * $p < 0.05$.

4. Discussion

In this study, we examined potential effects of two different social environments on endocrine and behavioural phenotypes across adolescence in male guinea pigs: males living in heterosexual pairs with additional social stimulation (PM+S) and males living in heterosexual pairs without additional social stimulation (PM-S). Regarding endocrine parameters, we found a significant treatment-by-time interaction effect for baseline cortisol levels (c0), with c0 values slightly decreasing over time in the PM+S group and increasing in the PM-S group. In contrast, no significant differences were found for baseline testosterone concentrations or increase in cortisol responsiveness after 1 h (c1) or 2 h (c2). However, repeatability of cortisol responsiveness differed between groups; with c1 being repeatable in PM+S males and c2 in PM-S males. Regarding behaviour, socially stimulated males displayed higher levels of sociopositive behaviour toward their female housing partner as well as towards unfamiliar conspecifics in the behavioural tests. No differences between social conditions were found in risk-taking behaviour.

4.1 Effects of social environment on endocrine phenotypes and their temporal stability

Baseline testosterone concentrations did not differ between the two social conditions. As outlined in the introduction, the classical “challenge” hypothesis proposes that increased social interactions with

conspecifics- especially agonistic interaction with other males as well as courtship and sexual interaction with females- can elevate testosterone levels in male vertebrates (Goymann et al., 2007; Hirschenhauser & Oliveira, 2006; Wingfield et al., 1990). However, more recent conceptual work has refined this view by assigning a more prominent role to interactions with reproductively active females (Goymann et al., 2019). Accordingly, variation in male androgen concentrations may be driven more strongly by mating opportunities than by competition among males (Goymann et al., 2019). In the present study, only one-third of the social stimulation sessions involved female conspecifics. The lower frequency of male-female encounters may therefore have limited their influence on baseline testosterone concentrations. Previous studies in guinea pigs further indicate that social stimulations can induce acute increases in circulating testosterone levels without affecting basal gonadal activity (Lürzel et al., 2010, 2011). Because testosterone in the present study was measured during CRTs, independently of social stimulation sessions, such short-term responses could not be detected. Sustained increases in basal testosterone may require more continuous exposure to reproductive social challenges, as under colony-housing conditions (Lürzel et al., 2010; Mutwill et al., 2020), whereas repeated brief encounters may have been insufficient to induce lasting changes.

In contrast, baseline cortisol showed a significant treatment-by-time interaction, indicating that the difference between the two social conditions changed over time. At CRT1, two weeks after the onset of social stimulation, PM+S males showed slightly higher baseline cortisol concentrations than PM-S males. This pattern was reversed over the experimental period, with PM-S males showing significantly higher baseline cortisol concentrations than PM+S males at CRT3, six weeks after the onset of social stimulation. However, c0 concentrations remained broadly stable and decreased only slightly over time in PM+S males, whereas c0 concentrations increased in PM-S males. As PM-S males were housed with a female partner and did not experience additional social challenges throughout the experimental phase, the observed increase in cortisol concentrations is unlikely to reflect increasing stress levels over time. In a study by Lürzel and colleagues, male guinea pigs also showed an increase in baseline cortisol concentrations from early to late adolescence which was interpreted as developmental effect (Lürzel et al., 2011). Similarly, studies in mice have shown that periadolescent males exhibit higher basal corticosterone levels compared to adults, again suggesting an age-related elevation glucocorticoid levels during adolescence (Adriani & Laviola, 2000; Laviola et al., 2002). This pattern may be linked to adolescence being a developmental phase characterised by complex alterations in brain structures and endocrine systems which also affect the regulation of the HPA axis (Brown & Spencer, 2013; Sisk & Zehr, 2005; Spear, 2000; Zimmermann, Kaiser, & Sachser, 2017). Still, it remains unclear why such an age-related increase was not observed in PM+S males. As discussed above, social stimulation can induce acute increases in circulating testosterone, which may inhibit HPA-axis (re)activity (Seale, Wood, Atkinson, Bate, et al., 2004; Seale, Wood, Atkinson, Harbuz, et al., 2004). Lürzel et al. (2010, 2011)

therefore suggested that repeated testosterone surges following social stimulation may have organisational effects on HPA responsiveness. Similar potential transient increases may have contributed to the attenuated age-related increase in baseline cortisol observed in PM+S males. Regarding HPA reactivity, no differences between the social conditions in cortisol responsiveness after 1 h (c1) or 2 h (c2) were found. Several guinea pig studies reported that different social experiences plastically shape cortisol responsiveness during adolescence (Lürzel et al., 2010, 2011; Sachser et al., 2013, 2018). However, adolescence itself represents a highly dynamic and plastic developmental phase (Sachser et al., 2013, 2018, 2020), and previous studies in rodents have shown that the impact of (social) challenges on HPA (re)activity is not uniform across adolescence but depends on the developmental stage at which these challenges occur (Barnum et al., 2012; Delville et al., 1998, 2003; Scharf et al., 2013; Torres Muñoz & Franklin, 2022). In the study by Lürzel and colleagues, social stimulation started later in adolescence than in the present study and the males also differed substantially in their rearing conditions (Lürzel et al., 2010). As social environments can already shape endocrine and behavioural phenotypes during juvenility (Gleske et al., 2026), differences in developmental timing and previous social experience may have contributed to the contrasting findings.

Although c1 and c2 concentrations did not differ between the social conditions, condition-specific repeatability patterns emerged. Cortisol responsiveness after 1 h (c1) was highly and significantly repeatable in PM+S males but not in PM-S males, whereas cortisol responsiveness after 2 h (c2) was highly and significantly repeatable in PM-S males but not in PM+S males. In guinea pigs, cortisol responsiveness after 1 hour reflects the speed of the stress response, whereas cortisol responsiveness after 2 hours indicates its magnitude (Rystrom, Wesseler, et al., 2024; Taff et al., 2022). One possible interpretation is that repeated exposure to short-term social challenges (social stimulation) in PM+S males might have stabilized individual differences in the initial phase of the stress response (c1, i.e., speed). This could be advantageous in an environment characterised by frequent social challenges, where a consistent and predictable initial stress response could facilitate efficient coping (Sangenstedt et al., 2017). At the same time, the absence of significant repeatability in the later phase of the stress response (c2, i.e., magnitude) may indicate that the magnitude of the stress response remains flexible in these males, allowing individuals to adjust their stress responsiveness according to the severity of a given environmental challenge (Sangenstedt et al., 2017). In contrast, in PM-S males, which were not exposed to such repeated social challenge, stable individual differences may have been more pronounced in the later phase of the response. In a more stable and less variable social environment, maintaining flexibility in the initial stress response while showing consistency in the overall magnitude of the stress response may be advantageous, as it could reduce unnecessary energetic costs (Sangenstedt et al., 2017). However, studies specifically addressing the repeatability of speed and

magnitude of stress response are currently lacking. These interpretations therefore remain speculative and do not yet allow conclusions about their potential adaptive significance.

As outlined in the introduction, social environment specific effects on the repeatability of endocrine traits have been discussed as potentially reflecting the differentiation of individualized social niches (Mutwill et al., 2023). A repeatability study conducted by Mutwill and colleagues, for example, found that in adolescent and adult male guinea pigs, baseline testosterone was repeatable in males housed in mixed-sex colonies but not in males housed in mixed-sex pairs (Mutwill et al., 2023). This finding was attributed to the occupation of individualized social niches by males under more complex colony-housing conditions (Mutwill et al., 2023). In this context, the distinct repeatability patterns of cortisol responsiveness observed in the present study may indicate that different social environments promote stability in different components of the stress response and could therefore reflect the differentiation of individualized social niches. Nevertheless, our findings align with a general pattern reported in a meta-analysis, showing that repeatability estimates tend to be higher for peak hormone levels than for baseline levels (Taff et al., 2018). The reason for this might be elevated hormone responses (e.g., through stress) capturing a more defined aspect of endocrine function, while baseline hormone levels can represent multiple different biological functions (Fanson & Biro, 2019; Hau et al., 2010).

4.2. Effects of social environment on (social) behaviour

Risk-taking behaviour was assessed in the step-down test to examine whether behavioural adjustments to the social environment extended beyond social contexts, as social niche conformance does not necessarily involve changes in social behaviour only (M. I. Kaiser et al., 2024). No differences between social conditions were detected. This contrasts with findings in other studies, where the social environment has been shown to influence behavioural domains such as risk-taking, for example in mice and rats (Mudra Rakshasa & Tong, 2020; Potřebić et al., 2021). One possible explanation is that risk-taking behaviour may be less sensitive to variation in the social environment due to domestication-related factors, as previous studies have reported higher levels of risk-taking behaviour in wild cavies compared to domesticated guinea pigs (Zipser et al., 2014). In their natural habitats, exploration is essential for wild cavies to access resources but inherently involves risk-taking due to high predation pressure (Asher et al., 2004; Zipser et al., 2014), whereas in domesticated guinea pigs, the constant availability of resources likely reduced the selection pressure for high levels of risk-taking behaviour (Zipser et al., 2014).

In contrast, PM+S males showed more sociopositive behaviour across all social contexts and more courtship behaviour towards females, indicating that prolonged social stimulation during adolescence was associated with increased sociopositive and courtship behaviour. This behavioural pattern could be advantageous in social environment characterised by a higher frequency and diversity of social

interactions. Under such conditions, behaviours resembling a queuing strategy may allow adolescent males to avoid reproductive competition until they are large enough to effectively challenge dominant males for mating access (Sachser et al., 2013, 2018; Zimmermann, Kaiser, & Sachser, 2017). At the same time, investing in social relationships with females can increase mating success, as female choice may favour males displaying higher amounts of sociopositive and courtship behaviour towards them (Mutwill et al., 2019; Sachser & Lick, 1989). Consistent with this, male guinea pigs less involved in male-male conflicts displayed more socio-sexual behaviour towards females and were more successful in female choice (Machatschke et al., 2008).

As outlined in the introduction, behavioural adjustments to the social environment can be interpreted within the framework of social niche conformance. In this context, higher levels of sociopositive and courtship behaviour observed in PM+S males may contribute to the avoidance of male-male conflict and the establishment of social relationships with females, thereby helping individuals adjust to a more variable social environment characterised by both increased potential mating opportunities and mating competition. Although such behavioural adjustments have previously been linked to endocrine mechanisms in guinea pigs (Sachser et al., 2013, 2018; Zimmermann, Kaiser, Hennessy, et al., 2017; Zimmermann, Kaiser, & Sachser, 2017), the absence of differences in baseline testosterone and HPA reactivity suggests that the behavioural effects observed here may have occurred independently of these mechanisms. Similar findings have been reported in zebra finches, where males adjusted their courtship and competitive behaviour to the social environment without corresponding changes in testosterone or corticosterone levels (Lilie et al., 2022). An alternative explanation is that the observed behavioural adjustment in this study was mediated by social learning processes. Social learning is known to play a crucial role during behavioural development in male guinea pigs (Sachser & Lick, 1989, 1991). Males raised in socially complex environments acquire appropriate behavioural responses through interactions with conspecifics, whereas limited social experience can result in learning deficiencies during behavioural development (Sachser & Lick, 1991). Recent findings in juvenile guinea pig males further support the idea that social experiences can shape behavioural phenotypes through learning processes (Gleske et al., 2026). Together, these findings suggest that the increased sociopositive and courtship behaviour in socially stimulated males in the present study could also reflect social learning rather than solely endocrine-mediated mechanisms.

Conclusion

The present study demonstrated that distinct social environments during adolescence influence both hormonal and behavioural phenotypes in male guinea pigs. The difference in baseline cortisol concentrations between the two social conditions changed over time. In addition, repeatability analyses revealed social condition-specific patterns in different components of cortisol responsiveness,

suggesting that adolescent social environments may not only influence endocrine phenotypes themselves but also the stability of inter-individual differences in these traits. Males exposed to additional social stimulation also showed increased sociopositive and courtship behaviour across different social contexts, which may reflect social niche conformance to a socially more variable and less predictable environment. Together, these findings emphasize adolescence as a dynamic developmental phase during which social experiences can shape behavioural and endocrine phenotypes.

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Supplementary material

From social experience to social behaviour: hormonal and behavioural phenotypes during adolescence in male guinea pigs

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Ethology

Supplementary material

Material and Methods: Ethogram

Table S1: Ethogram used for the observation of home enclosure behaviour. The abbreviation “FA” stands for “focus animal”, e.g., the experimental male.

Category	Behaviour	Description	Measurement
Courtship behaviour	Ano-genital licking	The FA stretches its snout towards or touches the female’s ano-genital region and lick or nuzzles the other female’s region. The distance between the two animals is less than one snout-width.	Frequency
Courtship behaviour	Rumba	The FA approaches the female slowly and visibly shifts its weight from one hind leg to the other and back, it can also move forward while doing so. This is often accompanied by a low purring noise. Behaviour ends when the FA stops for more than 3s.	Frequency
Sexual behaviour	Mating and mating attempt	The FA moves the forepart of its body onto the back of the female from behind. Subsequently, the FA either proceeds with pelvic thrusting (defined as fast, rhythmic movements of the lower body) or is prevented from doing so by the female.	Frequency
Sociopositive behaviour	Naso-nasal sniffing	The FA stretches its nose towards the female’s nose or snout. The distance between the two animals is less than one snout-width.	Frequency
Sociopositive behaviour	Naso-anal sniffing	The FA stretches its nose towards or touches the female’s anal region with its nose. The distance between the two animals is less than one snout-width.	Frequency

Table S2: Ethogram used for the male-female interaction test (MFIT). The abbreviation “FA” stands for “focus animal”, e.g., the experimental male.

Category	Behaviour	Description	Measurement
Courtship behaviour	Ano-genital licking	The FA stretches its snout towards or touches the female’s ano-genital region and lick or nuzzles the other female’s region. The distance between the two animals is less than one snout-width.	Frequency
Courtship behaviour	Rumba	The FA approaches the female slowly and visibly shifts its weight from one hind leg to the other and back, it can also move forward while doing so. This is often accompanied by a low purring noise. Behaviour ends when the FA stops for more than 3s.	Frequency
Sexual behaviour	Mating and mating attempt	The FA moves the forepart of its body onto the back of the female from behind. Subsequently, the FA either proceeds with pelvic thrusting (defined as fast, rhythmic movements of the lower body) or is prevented from doing so by the female.	Frequency
Sociopositive behaviour	First contact	The FA stretches its nose towards the female (at any body part) for the first time. The distance between the two animal is less than one snout-width.	Latency
Sociopositive behaviour	Naso-nasal sniffing	The FA stretches its nose towards the female’s nose or snout. The distance between the two animals is less than one snout-width.	Frequency
Sociopositive behaviour	Naso-anal sniffing	The FA stretches its nose towards or touches the female’s anal region with its nose. The distance between the two animals is less than one snout-width.	Frequency
Sociopositive behaviour	Being near female	The FAs head is within less than one head length of the female for at least 3s. Behaviour ends when not shown for at least 3s.	Duration

Table S3: Ethogram used for the social initiative test (SIT). The abbreviation “FA” stands for “focus animal”, e.g., the experimental male.

Phase	Category	Behaviour	Description	Measurement
Basket phase		Sniffing empty basket	The FA stretches its nose towards the empty basket. The distance between the FA and basket is less than one snout-width.	Frequency
Basket phase		Sniffing infant basket	The FA stretches its nose towards the infant basket. The distance between the FA and basket is less than one snout-width.	Frequency
Basket phase		Being near empty basket	The FAs head is within less than one head length of the empty basket for at least 3 seconds. Behaviour ends when not shown for at least 3s.	Duration
Basket phase		Being near infant basket	The FAs head is within less than one head length of the infant basket for at least 3 seconds. Behaviour ends when not shown for at least 3s.	Duration
No basket phase	Courtship behaviour	Ano-genital licking	The FA stretches its snout towards or touches the infant's ano-genital region and lick or nuzzles the other infant's region. The distance between the two animals is less than one snout-width.	Frequency
No basket phase	Courtship behaviour	Rumba	The FA approaches the infant slowly and visibly shifts its weight from one hind leg to the other and back, it can also move forward while doing so. This is often accompanied by a low purring noise. Behaviour ends when the FA stops for more than 3s.	Frequency
No basket phase	Sexual behaviour	Mating and mating attempt	The FA moves the forepart of its body onto the back of the infant from behind. Subsequently, the FA either proceeds with pelvic thrusting (defined as fast, rhythmic movements of the lower body) or is prevented from doing so by the infant.	Frequency

No basket phase	Sociopositive behaviour	First contact	The FA stretches its nose towards the infant (any body part) for the first time. The distance between the two animal is less than one snout-width.	Latency
No basket phase	Sociopositive behaviour	Naso-nasal sniffing	The FA stretches its nose towards the infant's nose or snout. The distance between the two animals is less than one snout-width.	Frequency
No basket phase	Sociopositive behaviour	Naso-anal sniffing	The FA stretches its nose towards or touches the infant's anal region with its nose. The distance between the two animals is less than one snout-width.	Frequency
No basket phase	Sociopositive behaviour	Being near infant	The FAs head is within less than one head length of the infant for at least 3s. Behaviour ends when not shown for at least 3s.	Duration

Results: Descriptive statistics

Table S4: Descriptive statistics for baseline cortisol (c0), increase in cortisol responsiveness after 1 hour (c1) and 2 hours (c2) of exposure to a novel environment and baseline testosterone (t). All hormones were measured as concentration in blood plasma (ng/ml).

Social condition	Hormone	Time point	n	mean	median	SD	min	max
PM+S	c0	CRT0	7	150.099	108.430	107.511	92.030	389.110
		CRT1	9	165.724	152.920	65.816	98.875	315.893
		CRT2	6	155.008	173.305	47.247	92.280	199.980
		CRT3	6	137.668	142.056	46.030	57.500	187.155
	c1	CRT0	7	677.405	575.795	186.908	542.195	1030.205
		CRT1	9	702.344	717.743	137.137	499.308	897.080
		CRT2	6	795.075	759.454	160.506	589.775	1037.135
		CRT3	6	767.320	756.468	105.843	626.950	889.895
	c2	CRT0	7	1033.455	930.670	234.253	810.555	1467.880
		CRT1	9	920.504	836.740	188.361	648.800	1196.530
		CRT2	6	1049.784	1015.504	165.196	855.150	1347.360
		CRT3	6	968.482	952.219	210.597	634.910	1265.245
	t	CRT0	10	4.432	3.770	2.364	1.090	9.180
		CRT1	10	3.663	3.580	1.432	1.460	6.430
		CRT2	10	3.563	3.173	1.255	2.340	6.300
		CRT3	9	3.554	3.408	0.847	2.370	5.130
PM-S	c0	CRT0	9	163.348	149.570	83.864	44.260	320.620
		CRT1	6	133.484	116.268	62.463	74.688	243.730
		CRT2	4	185.590	185.690	45.501	130.250	240.730
		CRT3	7	197.902	173.200	80.834	124.020	356.445
	c1	CRT0	9	612.711	635.595	222.035	315.655	955.415
		CRT1	6	819.545	865.561	208.619	436.295	1038.305
		CRT2	4	780.841	807.728	212.614	497.010	1010.900
		CRT3	6	665.239	778.128	321.084	44.805	923.915
	c2	CRT0	9	932.778	991.030	333.408	342.405	1361.440
		CRT1	6	1118.212	1140.649	285.154	586.495	1374.790
		CRT2	4	1088.435	1082.203	202.359	850.135	1339.200
		CRT3	6	1072.110	1054.218	224.583	801.005	1360.815
	t	CRT0	9	4.970	4.650	2.504	1.350	8.690
		CRT1	10	3.639	3.810	1.553	0.970	6.192
		CRT2	10	3.392	3.568	1.083	1.880	5.130
		CRT3	10	3.619	3.395	0.896	2.540	4.920

Table S5: Descriptive statistics for behavioural parameters assessed in the home enclosure towards the female housing partner. All behaviours were measured as frequencies per hour.

Social condition	Behaviour	Time point	n	mean	median	SD	min	max
PM+S	Sociopositive	Phase 1	10	23.4	16.5	21.282	4	76
		Phase 2	10	20.4	17.5	11.108	13	50
		Phase 3	10	21.4	16.5	16.507	4	52
	Courtship	Phase 1	10	3.8	2.5	4.894	0	16
		Phase 2	10	3.2	1	4.131	0	11
		Phase 3	10	1.3	1	1.494	0	5
	Sexual	Phase 1	10	0.1	0	0.316	0	1
		Phase 2	10	0.1	0	0.316	0	1
		Phase 3	10	0	0	0.000	0	0
PM-S	Sociopositive	Phase 1	10	16.3	14	10.853	5	39
		Phase 2	10	11.7	7.5	11.076	0	28
		Phase 3	10	9.9	7	10.049	2	34
	Courtship	Phase 1	10	1.2	1	1.229	0	3
		Phase 2	10	0.9	0.5	1.197	0	3
		Phase 3	10	1.4	1	1.713	0	5
	Sexual	Phase 1	10	0.1	0	0.316	0	1
		Phase 2	10	0	0	0.000	0	0
		Phase 3	10	0.1	0	0.316	0	1

Table S6: Descriptive statistics for the step-down test.

Social condition	Variable	n	mean	median	SD	min	max
PM+S	Latency to step down	10	409.8	319	350.845	1	900
PM-S	Latency to step down	10	515.5	724.5	435.177	1	900

Table S7: Descriptive statistics for the male-female interaction test.

Social condition	Variable	n	mean	median	SD	min	max
PM+S	Latency first contact female	10	186.8	9.5	566.834	1	1800
	Time spent near female	10	949	1052.5	385.698	57	1414
	Sociopositive behaviour	10	148.4	154.5	77.04	0	271
	Courtship behaviour	10	22.1	21.5	11.532	0	39
	Sexual behaviour	10	11.5	1	25.33	0	79
PM-S	Latency first contact female	9	188.111	6	462.504	2	1406
	Time spent near female	9	874.778	861	331.512	245	1339
	Sociopositive behaviour	9	79.333	90	43.824	10	154
	Courtship behaviour	9	12	10	6.708	4	24
	Sexual behaviour	9	16.889	1	30.945	0	93

Table S8: Descriptive statistics for the social initiative test.

Social condition	Variable	n	mean	median	SD	min	max
PM+S	Sniffing empty basket	9	15.444	17	6.784	3	22
	Sniffing infant basket	9	35.889	39	12.82	15	53
	Time spent near empty basket	9	83.111	105	46.948	20	139
	Time spent near infant basket	9	321.333	267	156.179	121	644
	Latency first contact infant	9	31.444	20	39.627	1	132
	Time spent near infant	9	463	459	260.379	22	806
	Sociopositive behaviour	9	63.333	67	31.153	3	114
	Courtship behaviour	9	10.667	11	5.679	0	18
	Sexual behaviour	9	1	0	2	0	6
PM-S	Sniffing empty basket	10	17.9	16.5	8.293	3	28
	Sniffing infant basket	10	40.1	44.5	16.381	4	57
	Time spent near empty basket	10	89.7	63.5	78.014	3	248
	Time spent near infant basket	10	261.4	285	132.831	33	440
	Latency first contact infant	10	129.1	20.5	276.444	1	900
	Time spent near infant	10	543.7	620	255.198	0	815
	Sociopositive behaviour	10	30.5	37	17.018	0	50
	Courtship behaviour	10	7.9	5.5	8.13	0	28
	Sexual behaviour	10	0.6	0	1.265	0	3

Results: Wilcoxon test for treatment comparisons of hormone concentrations at CRT0

Table S9: Wilcoxon rank-sum tests of hormone concentrations calculated for the first cortisol response test (CRT) conducted before treatment (PM+S; PM-S).

Wilcoxon rank-sum test (CRT0)	W	r	p-value
Baseline cortisol	42	0.237	0.290
Increase in cortisol responsiveness, 1h	26	0.118	0.597
Increase in cortisol responsiveness, 2h	31	0	1
Baseline testosterone	51	0.1	0.653

Results: Linear mixed-effects models for hormone concentrations

Table S10: Model comparisons based on likelihood ratio tests for linear mixed-effects models used to analyse hormone concentrations. For each hormone (baseline cortisol (c0), increase in cortisol responsiveness after 1 hour of exposure to a novel environment (c1), increase in cortisol responsiveness after 2 hours of exposure to a novel environment (c2) and baseline testosterone (t)), models with and without the interaction between treatment (social condition) and time (CRT) were compared, followed by comparisons of random-effect structures. Random-effect structures compared models including individual ID as random effect with models including individual ID nested within pair ID. All models additionally included body weight as fixed covariate. ($p < 0.05$) results are indicated in bold.

Hormone	Comparison	Model1	Model2	AIC model1	AIC model2	χ^2	Df	p-value
c0	Interaction comparison	CRT + Treatment + (1 PairID/ID)	CRT*Treatment + (1 PairID/ID)	426.481	422.320	8.161	2	0.017
c1	Interaction comparison	CRT + Treatment + (1 PairID/ID)	CRT*Treatment + (1 PairID/ID)	504.748	506.024	2.724	2	0.256
c2	Interaction comparison	CRT + Treatment + (1 PairID/ID)	CRT*Treatment + (1 PairID/ID)	502.879	505.885	0.994	2	0.608
testosterone	Interaction comparison	CRT + Treatment + (1 PairID/ID)	CRT*Treatment + (1 PairID/ID)	190.549	194.512	0.037	2	0.982
c0	Random-effect comparison	CRT*Treatment + (1 ID)	CRT*Treatment + (1 PairID/ID)	375.153	377.043	0.110	1	0.740
c1	Random-effect comparison	CRT + Treatment + (1 ID)	CRT + Treatment + (1 PairID/ID)	462.238	464.237	0.001	1	0.979
c2	Random-effect comparison	CRT + Treatment + (1 ID)	CRT + Treatment + (1 PairID/ID)	461.289	461.856	1.433	1	0.231
testosterone	Random-effect comparison	CRT + Treatment + (1 ID)	CRT + Treatment + (1 PairID/ID)	201.691	203.691	0.000	1	1.000

Table S11: Model summary from linear mixed-effects model used to analyse baseline cortisol (N = 38). The model included the interaction between treatment (social condition) and time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Baseline cortisol	Estimate	Std. error	[95% CI]	t-value	p-value	R ²
					Full model: Marginal R ²	0.332
					Full model: Conditional R ²	0.393
Intercept	107.550	23.176	[60.249, 154.851]	4.640	< 0.001	
Fixed effects						
Treatment (social condition)	39.402	28.268	[-18.272, 97.075]	1.394	0.173	0.057
CRT1- CRT2 (time)	84.091	35.223	[11.747, 156.436]	2.387	0.024	0.160
CRT1- CRT3 (time)	123.500	33.840	[54.384, 192.616]	3.650	0.001	0.290
Body weight	-0.510	0.157	[-0.837,-0.184]	-3.242	0.004	0.269
Treatment*CRT1-CRT2	-69.042	43.185	[-158.289, 20.205]	-1.599	0.123	0.077
Treatment*CRT1-CRT3	-110.785	39.731	[-193.198,-28.371]	-2.788	0.011	0.182

Table S12: Model summary from linear mixed-effects model used to analyse increase in cortisol responsiveness after 1 hour of exposure to a novel environment (N = 37). The model included treatment (social condition), time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Increase in Cortisol responsiveness, 1h	Estimate	Std. error	[95% CI]	t-value	p-value	R ²
					Full model: Marginal R ²	0.052
					Full model: Conditional R ²	0.341
Intercept	781.122	72.804	[630.321, 931.923]	10.729	< 0.001	
Fixed effects						
Treatment (social condition)	-24.598	77.811	[-191.442, 142.245]	-0.316	0.757	< 0.001
CRT1- CRT2 (time)	21.911	79.907	[-141.270, 185.091]	0.274	0.786	< 0.001
CRT1- CRT3 (time)	-81.553	92.929	[-271.378, 108.273]	-0.878	0.387	0.030
Body weight	0.517	0.612	[-0.756, 1.789]	0.844	0.408	0.037

Table S13: Model summary from linear mixed-effects model used to analyse increase in cortisol responsiveness after 2 hours of exposure to a novel environment (N = 37). The model included treatment (social condition), time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Increase in Cortisol responsiveness, 2h	Estimate	Std. error	[95% CI]	t-value	p-value	R ²
					Full model: Marginal R ²	0.139
					Full model: Conditional R ²	0.680
Intercept	1114.074	84.386	[936.580, 1291.568]	13.202	< 0.001	
Fixed effects						
Treatment (social condition)	-153.490	98.039	[-365.781, 58.801]	-1.566	0.142	0.086
CRT1- CRT2 (time)	16.600	75.343	[-137.034, 170.234]	0.220	0.827	0.008
CRT1- CRT3 (time)	-58.170	98.207	[-259.291, 142.951]	-0.592	0.558	0.001
Body weight	0.653	0.697	[-0.789, 2.095]	0.938	0.358	0.007

Table S14: Model summary from linear mixed-effects model used to analyse baseline testosterone (N = 59). The model included treatment (social condition), time (CRT) and body weight as fixed effects, with individual ID as random effect. CRT1 (time) and PM-S (treatment) were set as reference level by default.

Baseline testosterone	Estimate	Std. error	[95% CI]	t-value	p-value	R ²
					Full model: Marginal R ²	0.077
					Full model: Conditional R ²	0.374
Intercept	3.826	0.345	[3.121, 4.531]	11.084	< 0.001	
Fixed effects						
Treatment (social condition)	-0.056	0.393	[-0.883, 0.772]	-0.142	0.889	< 0.001
CRT1- CRT2 (time)	-0.511	0.359	[-1.231, 0.210]	-1.421	0.161	0.031
CRT1- CRT3 (time)	-0.604	0.450	[-1.509, 0.302]	-1.341	0.186	0.035
Body weight	0.005	0.003	[-0.001, 0.010]	1.744	0.095	0.082

Results: Multiple comparisons of linear mixed-effects models of hormone concentrations

Table S15: Multiple comparisons (Tukey's) of linear mixed-effects model to determine effects of treatment (social condition), time (CRT) and treatment*time interaction on baseline cortisol. Significant ($p < 0.05$) results are indicated in bold.

Baseline cortisol	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
CRT1	-39.402	28.540	30.697	[-97.633, 18.830]	-1.381	0.177
CRT2	29.641	35.427	30.976	[-42.615, 101.896]	0.837	0.409
CRT3	71.383	30.279	30.911	[9.621, 133.146]	2.357	0.025
Pair-wise comparison (between time points)						
CRT 1- CRT 2 (PM-S)	-84.091	36.311	25.881	[-174.345, 6.163]	-2.316	0.071
CRT 1- CRT 3 (PM-S)	-123.500	34.531	29.792	[-208.658, -38.342]	-3.577	0.003
CRT 2- CRT 3 (PM-S)	-39.409	34.394	26.842	[-124.715, 45.897]	-1.146	0.495
CRT 1- CRT 2 (PM+S)	-15.049	28.760	25.184	[-86.652, 56.554]	-0.523	0.861
CRT 1- CRT 3 (PM+S)	-12.715	30.621	30.044	[-88.199, 62.768]	-0.415	0.910
CRT 2- CRT 3 (PM+S)	2.334	30.500	21.967	[-74.293, 78.960]	0.077	0.997
Interaction contrasts (social condition * time point)						
CRT1- CRT2	69.042	44.179	22.607	[-22.437, 160.521]	1.563	0.132
CRT1- CRT3	110.785	40.313	21.024	[26.955, 194.614]	2.748	0.012
CRT2- CRT3	41.742	45.179	22.864	[-51.749, 135.234]	0.924	0.365

Table S16: Multiple comparisons (Tukey's) of linear mixed-effects model to determine effects of treatment (social condition) and time (CRT) on increase in cortisol responsiveness after 1 hour of exposure to a novel environment.

Increase in Cortisol responsiveness, 1h	Estimate	Std. error	df	[95% CI]	t-value	p-value
Social condition	24.598	78.277	13.312	[-144.106, 193.303]	0.314	0.758
CRT (Time)						
CRT 1- CRT 2	-21.911	81.309	29.874	[-222.403, 178.582]	-0.269	0.961
CRT 1- CRT 3	81.553	94.765	29.658	[-152.207, 315.313]	0.861	0.669
CRT 2- CRT 3	103.463	80.537	27.471	[-96.025, 302.951]	1.285	0.416

Table S17: Multiple comparisons (Tukey's) of linear mixed-effects model to determine effects of treatment (social condition) and time (CRT) on increase in cortisol responsiveness after 2 hours of exposure to a novel environment.

Increase in Cortisol responsiveness, 2h	Estimate	Std. error	df	[95% CI]	t-value	p-value
Social condition	153.490	98.261	13.816	[-57.524, 364.503]	1.562	0.141
CRT (Time)						
CRT 1- CRT 2	-16.600	77.071	31.256	[-206.210, 173.009]	-0.215	0.975
CRT 1- CRT 3	58.170	101.572	28.605	[-192.862, 309.202]	0.573	0.836
CRT 2- CRT 3	74.770	72.659	27.533	[-105.182, 254.722]	1.029	0.565

Table S18: Multiple comparisons (Tukey's) of linear mixed-effects model to determine effects of treatment (social condition) and time (CRT) on baseline testosterone.

Baseline testosterone	Estimate	Std. error	df	[95% CI]	t-value	p-value
Social condition	0.056	0.393	16.970	[-0.774, 0.885]	0.142	0.889
CRT (Time)						
CRT 1- CRT 2	0.511	0.361	53.141	[-0.359, 1.381]	1.415	0.341
CRT 1- CRT 3	0.604	0.454	46.676	[-0.495, 1.702]	1.330	0.386
CRT 2- CRT 3	0.093	0.337	48.843	[-0.722, 0.908]	0.276	0.959

Results: Adjusted repeatability analysis of hormone concentrations

Table S19: Adjusted repeatability analysis of linear mixed-effects models of baseline cortisol (c0), increase in cortisol responsiveness after 1 hour of exposure to a novel environment (c1), increase in cortisol responsiveness after 2 hours of exposure to a novel environment (c2) and baseline testosterone (t). Significant ($p < 0.05$) results are indicated in bold.

Repeatability	PM-S				PM+S			
	Std. error	[95% CI]	R	p-value	Std. error	[95% CI]	R	p-value
c0	0.270	[0, 0.833]	0.124	0.401	0.220	[0, 0.685]	0.076	0.392
c1	0.292	[0, 0.877]	0.121	0.432	0.185	[0.233, 0.945]	0.721	< 0.001
c2	0.164	[0.321, 0.980]	0.782	0.007	0.248	[0, 0.864]	0.418	0.056
t	0.203	[0, 0.732]	0.346	0.076	0.202	[0, 0.669]	0.244	0.153

Results: Generalized linear mixed-effects models for behaviour

Table S20: Model comparisons based on likelihood ratio tests for generalized mixed-effects models used to analyse behavioural counts. For each behaviour (sociopositive behaviour, courtship behaviour) models with and without the interaction between treatment (social condition) and time (phase) were compared, followed by comparisons of random-effect structures. Random-effect structures compared models including individual ID as random effect with models including individual ID nested within pair ID.

Behaviour	Comparison	Model1	Model2	AIC model1	AIC model2	χ^2	Df	p-value
Sociopositive	Interaction comparison	Phase + Treatment + (1 PairID/ID)	Phase*Treatment + (1 PairID/ID)	464.208	467.467	0.741	2	0.690
Courtship	Interaction comparison	Phase + Treatment + (1 PairID/ID)	Phase*Treatment + (1 PairID/ID)	232.963	233.843	3.120	2	0.210
Sociopositive	Random-effect comparison	Phase + Treatment + (1 ID)	Phase + Treatment + (1 PairID/ID)	462.208	464.208	0.000	1	1.000
Courtship	Random-effect comparison	Phase + Treatment + (1 ID)	Phase + Treatment + (1 PairID/ID)	230.963	232.963	0.000	1	1.000

Table S21: Model summary from generalized linear mixed-effects model used to analyse sociopositive behaviour (N = 60). The model included treatment (social condition) and time (phase) as fixed effects and individual ID as random effect. Phase 1 (time) and PM-S (treatment) were set as reference level by default.

Sociopositive behaviour	Estimate	Std. error	[95% CI]	z-value	p-value	R ²
					<i>Full model: Marginal R²</i>	0.173
					<i>Full model: Conditional R²</i>	0.190
Intercept	2.686	0.214	[2.266, 3.105]	12.534	< 0.001	
Fixed effects						
Treatment (social condition)	0.566	0.211	[0.152, 0.981]	2.678	0.007	0.103
Phase 1- Phase 2 (time)	-0.234	0.244	[-0.711, 0.244]	-0.959	0.337	0.013
Phase 1- Phase 3 (time)	-0.287	0.242	[-0.762, 0.187]	-1.186	0.236	0.016

Table S22: Model summary from generalized linear mixed-effects model used to analyse courtship behaviour (N = 60). The model included treatment (social condition) and time (phase) as fixed effects and individual ID as random effect. Phase 1 (time) and PM-S (treatment) were set as reference level by default.

Courtship behaviour	Estimate	Std. error	[95% CI]	z-value	p-value	R ²
					<i>Full model: Marginal R²</i>	0.172
					<i>Full model: Conditional R²</i>	0.247
Intercept	0.347	0.397	[-0.432, 1.125]	0.873	0.383	
Fixed effects						
Treatment (social condition)	0.820	0.371	[0.092, 1.547]	2.209	0.027	0.076
Phase 1- Phase 2 (time)	-0.213	0.403	[-1.003, 0.577]	-0.528	0.597	0.004
Phase 1- Phase 3 (time)	-0.485	0.423	[-1.313, 0.344]	-1.146	0.252	0.028

Results: Multiple comparisons of generalized linear mixed-effects models of behaviour

Table S23: Multiple comparisons (Tukey's) of generalized linear mixed-effects model to determine effects of treatment (social condition) and time (phase) on sociopositive behaviour. Significant ($p < 0.05$) results are indicated in bold.

Sociopositive behaviour	Estimate	Std. error	df	[95% CI]	z-value	p-value
Social condition	-0.566	0.211	Inf	[-0.981, -0.152]	-2.678	0.007
Phase (Time)						
Phase 1- Phase 2	0.234	0.244	Inf	[-0.337, 0.805]	0.959	0.603
Phase 1- Phase 3	0.287	0.242	Inf	[-0.280, 0.855]	1.186	0.462
Phase 2- Phase 3	0.054	0.245	Inf	[-0.521, 0.628]	0.219	0.974

Table S24: Multiple comparisons (Tukey's) of generalized linear mixed-effects model to determine effects of treatment (social condition) and time (phase) on courtship behaviour. Significant ($p < 0.05$) results are indicated in bold.

Courtship behaviour	Estimate	Std. error	df	[95% CI]	z-value	p-value
Social condition	-0.820	0.371	Inf	[-1.547, -0.092]	-2.209	0.027
Phase (Time)						
Phase 1 - Phase 2	0.213	0.403	Inf	[-0.732, 1.157]	0.528	0.857
Phase 1 - Phase 3	0.485	0.423	Inf	[-0.507, 1.476]	1.146	0.486
Phase 2 - Phase 3	0.272	0.428	Inf	[-0.730, 1.274]	0.635	0.801

Results: Statistical tests for behavioural tests (SDT, MFIT, SIT)

Table S25: Fisher's exact test and Wilcoxon rank-sum test results for the step-down test (SDT) (N = 20).

Fisher's exact test (SDT)	Odds ratio	[95% CI]	p-value
Proportion of individuals stepping down	3.719	[0.405, 53.810]	0.350
Wilcoxon rank-sum test (SDT)	W	r	p-value
Latency to step down	11	0.347	0.211

Table S26: Wilcoxon rank-sum test and Welch's t-test results for the male-female interaction test (MFIT) (N = 19). Significant ($p < 0.05$) results are indicated in bold.

Wilcoxon rank-sum test (MFIT)	W	r	p-value
Latency first contact with female	46.5	0.019	0.935
Sexual behaviour	48	0.048	0.833
Welch's t-test (MFIT)	t	Cohen's d	p-value
Time spent near female	-0.45	0.205	0.658
Sociopositive behaviour	-2.43	1.086	0.029
Courtship behaviour	-2.36	1.055	0.032

Table S27: Wilcoxon rank-sum test and Welch's t-test results for the social initiative test (SIT) during the no basket phase (N = 19). Significant ($p < 0.05$) results are indicated in bold.

Wilcoxon rank-sum test (SIT no basket phase)	W	r	p-value
Latency first contact with infant	47.5	0.038	0.870
Welch's t-test (SIT no basket phase)	t	Cohen's d	p-value
Time spent near infant	0.681	0.313	0.505
Sociopositive behaviour	-2.807	1.329	0.016
Courtship behaviour	-0.867	0.391	0.399

Results: Model summaries of (generalized) linear mixed-effects models for the social initiative test (SIT) during the basket phase

Table S28: Model summary from generalized linear mixed-effects model used to analyse basket sniffing in the social initiative test (SIT) during the basket phase (N = 19). The model included the interaction between treatment (social condition) and basket type (empty or infant) with individual ID as random effect. Empty basket (basket type) and PM-S (treatment) were set as reference level by default.

Sniffing empty and infant basket (SIT)	Estimate	Std. error	[95% CI]	z-value	p-value	R ²
					<i>Full model: Marginal R²</i>	0.427
					<i>Full model: Conditional R²</i>	0.915
Intercept	2.784	0.162	[2.466, 3.101]	17.182	< 0.001	
Fixed effects						
Treatment (social condition)	-0.103	0.236	[-0.566, 0.360]	-0.437	0.662	0.006
Basket type	0.807	0.090	[0.631, 0.982]	9.005	< 0.001	0.343
Treatment*Basket type	0.037	0.135	[-0.228, 0.301]	0.271	0.786	0.002

Table S29: Model summary from linear mixed-effects model used to analyse the duration of time spent near the basket in the social initiative test (SIT) during the basket phase (N = 19). Data was log-transformed. The model included the interaction between treatment (social condition) and basket type (empty or infant) with individual ID as random effect. Empty basket (basket type) and PM-S (treatment) were set as reference level by default.

Time spent near empty and infant basket (SIT)	Estimate	Std. error	[95% CI]	t-value	p-value	R ²
					<i>Full model: Marginal R²</i>	0.400
					<i>Full model: Conditional R²</i>	0.744
Intercept	4.045	0.277	[3.474, 4.615]	14.583	< 0.001	
Fixed effects						
Treatment (social condition)	0.149	0.403	[-0.680, 0.978]	0.370	0.714	0.004
Basket type	1.321	0.256	[0.781, 1.862]	5.156	< 0.001	0.250
Treatment*Basket type	0.153	0.372	[-0.632, 0.939]	0.411	0.686	0.002

Results: Multiple comparisons of (generalized) linear mixed-effects models for the social initiative test (SIT) during the basket phase

Table S30: Multiple comparisons (Tukey's) of generalized linear mixed-effects model to determine effects of treatment (social condition), basket type (infant or empty) and treatment*basket type interaction on basket sniffing in the social initiative test (SIT) during the basket phase. Significant ($p < 0.05$) results are indicated in bold.

Sniffing empty and infant basket during SIT	Estimate	Std. error	df	[95% CI]	z-value	p-value
Pair-wise comparison (between social conditions)						
Empty basket	0.103	0.236	Inf	[-0.360, 0.566]	0.437	0.662
Infant basket	0.067	0.221	Inf	[-0.366, 0.499]	0.302	0.763
Pair-wise comparison (between basket types)						
PM-S	-0.807	0.090	Inf	[-0.982,-0.631]	-9.005	< 0.001
PM+S	-0.843	0.101	Inf	[-1.041,-0.645]	-8.345	< 0.001
Interaction contrasts (social condition * basket type)	0.037	0.135	Inf	[-0.228, 0.301]	0.271	0.786

Table S31: Multiple comparisons (Tukey's) of linear mixed-effects model to determine effects of treatment (social condition), basket type (infant or empty) and treatment*basket type interaction on the duration of time spent near baskets in the social initiative test (SIT) during the basket phase. Significant ($p < 0.05$) results are indicated in bold.

Time spent near empty and infant basket during SIT	Estimate	Std. error	df	[95% CI]	t-value	p-value
Pair-wise comparison (between social conditions)						
Empty basket	-0.149	0.403	25.591	[-0.978, 0.680]	-0.370	0.714
Infant basket	-0.302	0.403	25.591	[-1.131, 0.527]	-0.750	0.460
Pair-wise comparison (between basket types)						
PM-S	-1.321	0.256	17	[-1.862,-0.781]	-5.156	< 0.001
PM+S	-1.474	0.270	17	[-2.044,-0.904]	-5.458	< 0.001
Interaction contrasts (social condition * basket type)	0.153	0.372	17	[-0.632, 0.939]	0.411	0.686

Results: Figures for behavioural tests (SDT, MFIT, SIT)

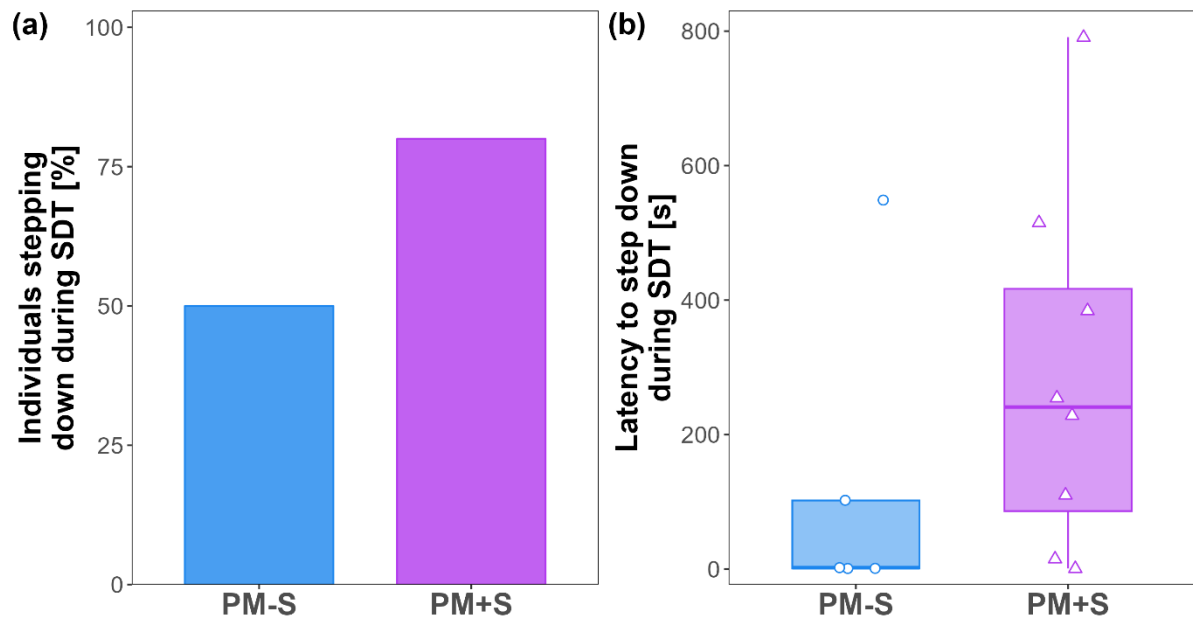


Figure S1: Step-down test (SDT). **(a)** Percentage of individuals stepping down from the platform within the maximum test duration (900 s) and **(b)** latency to step down from the platform for individuals that stepped down. Males were either additionally socially stimulated (PM+S) or not (PM-S). In **(a)**, bars represent the proportion of individuals per group. In **(b)**, boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range

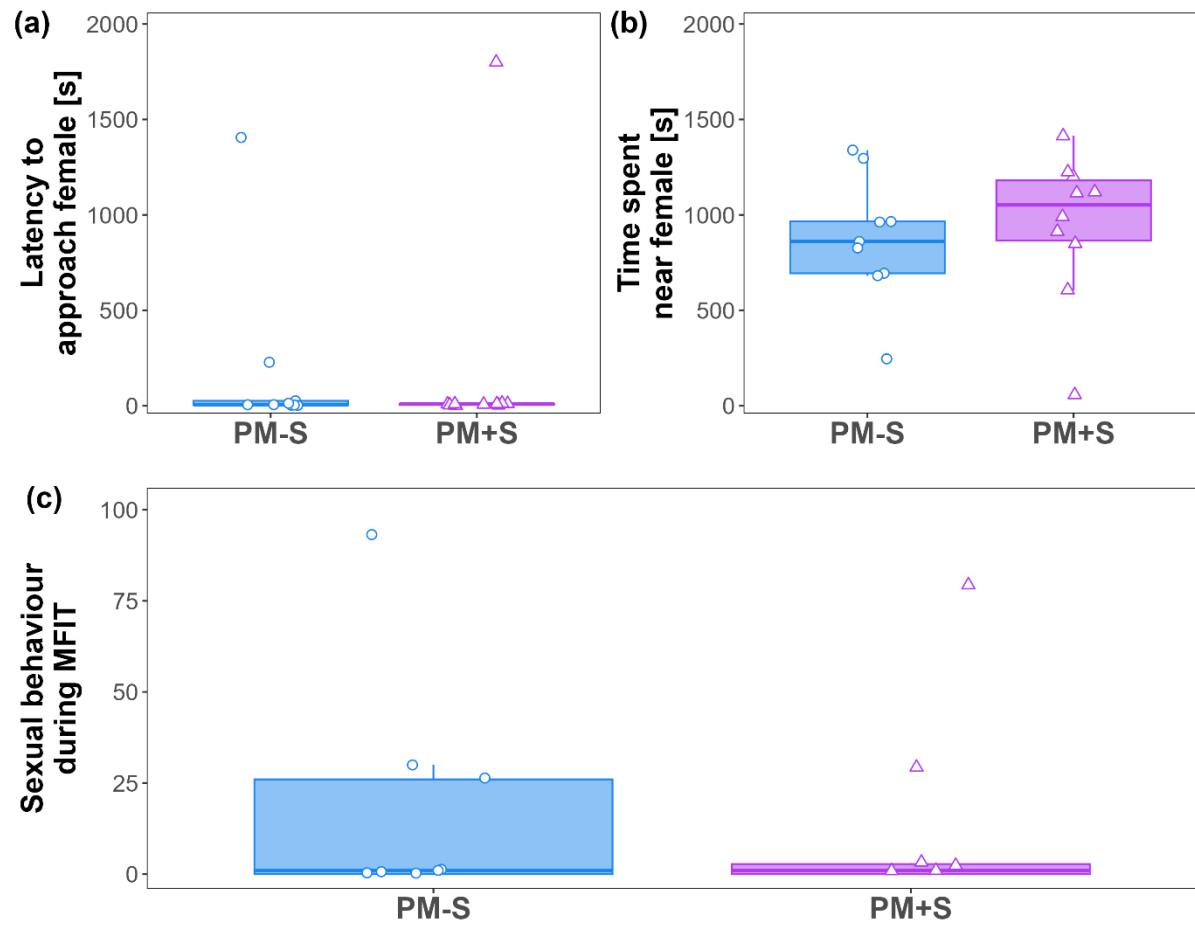


Figure S2: Male-female interaction test (MFIT). **(a)** Latency to first contact with female, **(b)** time spent near female and **(c)** behavioural counts of sexual behaviour towards female. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range

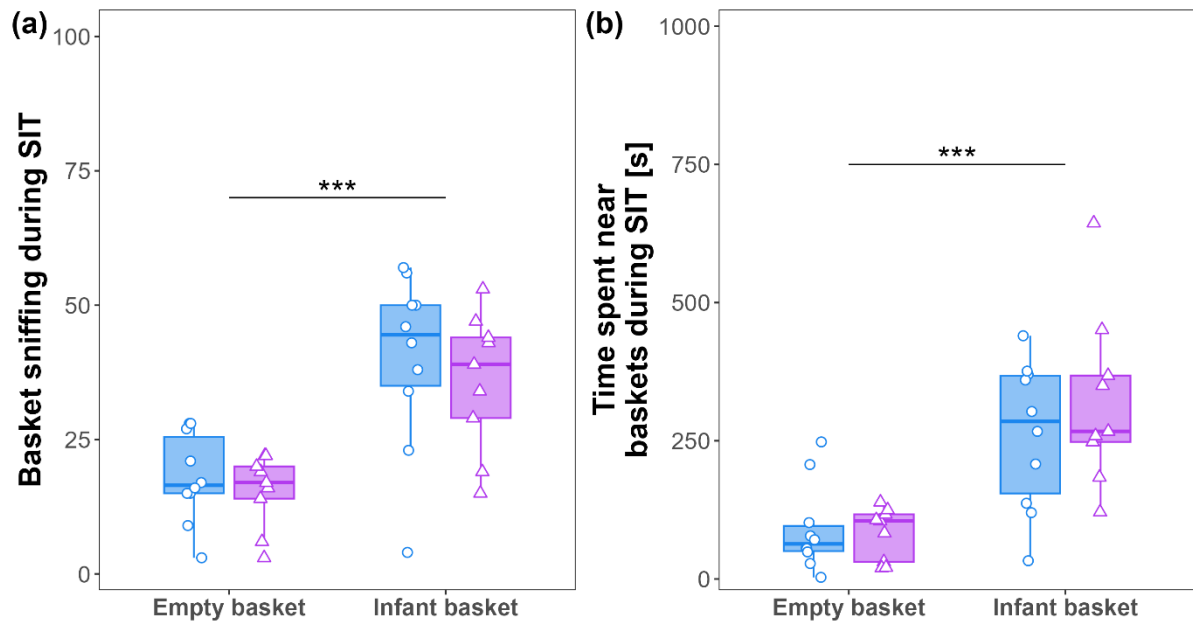


Figure S3: Social initiative test (SIT), basket phase. **(a)** Sniffing of the empty and infant basket and **(b)** time spent near the empty and infant basket. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range. *** $p < 0.001$.

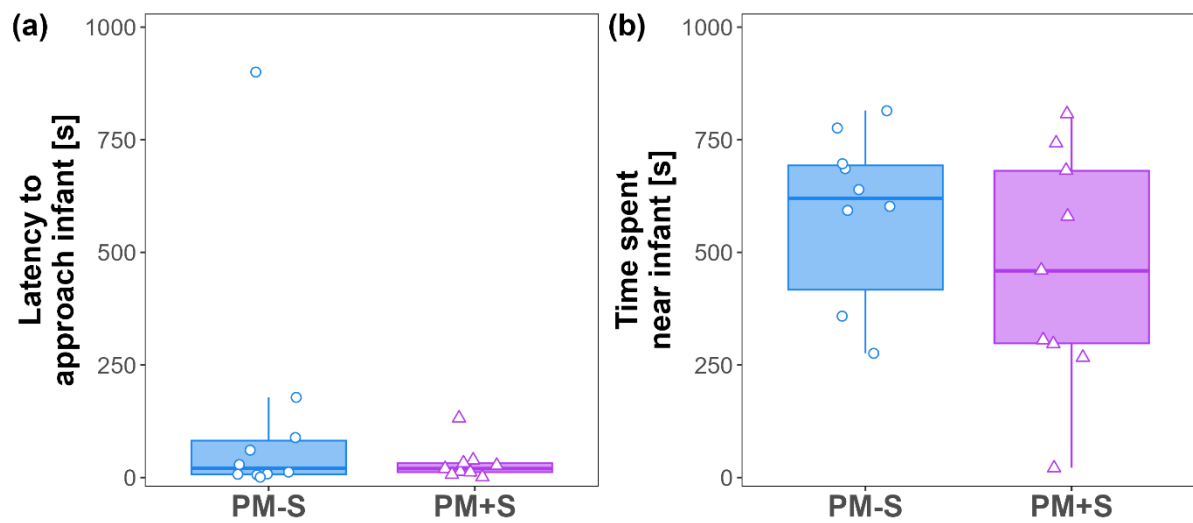


Figure S4: Social initiative test (SIT), no basket phase. **(a)** Latency to first contact with infant and **(b)** time spent near infant. Males were either additionally socially stimulated (PM+S) or not (PM-S). Boxplots show raw data with individual data points. Boxes represent the interquartile range, horizontal lines in boxes indicate medians and whiskers extend to the most extreme values within 1.5 times the interquartile range.